 REMARKS

Claims 1-20 are currently pending in the application. By this amendment, claims 1-7 and 9-15 have been amended and new claims 17-20 have been added for the Examiner's consideration. The foregoing separate sheets marked as "Listing of Claims" shows all the claims in the application, with an indication of the current status of each.

Claim Rejections: 35 USC § 112

Second Paragraph Rejections

Claims 1-7, 11, 12, 14 and 15 stand rejected under 35 USC § 112, second paragraph, as being indefinite.

Examiner has pointed out that claims 2-6, 12, and 15 do not end in a period. Applicant has hereby amended claims 2-6, 12 and 15 to include a period. Applicant submits that this amendment does not introduce new matter, being entirely formal in nature. Applicant respectfully requests entry of this amendment, and withdrawal of this portion of the rejection.

Examiner states that claims 1, 7, 11 and 14 define "X" as a "saturated or unsaturated C1 to C12 carbon chain", but that it is not possible for a C1 to be unsaturated. Claims 1, 7, 11 and 14 have hereby been amended to recite that X is CH_2 or a saturated or unsaturated C_2 carbon chain. Applicant submits that this amendment adds no new matter, being undertaken simply to correct a error in chemical nomenclature. Applicant therefore respectfully requests entry of this amendment, and withdrawal of this portion of the rejection.

Examiner has requested removal of the word "generic" from claims 11 and 14. Claims 11 and 14 have hereby been amended by the removal of this word. Applicant submits that removal of this single word does not add any new matter, and respectfully requests entry of the amendment and withdrawal of this portion of the rejection.

First Paragraph Rejection

Claims 7-16 stand rejected under 35 USC 112, second paragraph, as not enabled by the specification. The Examiner states that the specification of the application is enabling for the treatment of vomiting, nausea, and appetite loss, but not for the treatment of conditions or disorders related to cannabinoid-regulated systems in general. Examiner has provided a reference (Mechoulam et al.) that teaches that the regulation of cannabinoid systems is useful for the

treatment of vomiting, nausea, and appetite loss. Claim 7 has hereby been amended to recite the specific conditions of vomiting, nausea, and appetite loss as some of those that may be treated by the methods of the invention.

Applicants further submit that other conditions exist which were disclosed in the instant specification (e.g. on page 12 at lines 8-17 and on page 14 lines 7-24) and are generally known to be amenable to treatment with cannabinoids. In support of this, Applicant herewith encloses the following references:

1) CB1 antagonists to treat craving for addictive drugs: Le Foll and Goldberg, 2005, *Journal of Pharmacology and Experimental Therapeutics*, 312: 875-883;
2) CB1 antagonists as appetite suppressants for the treatment of obesity: Van Gaal et al. 2005, *The Lancet*, 365:13891397; Le Fur et al., *International Cannabinoid Research Society*, June 28-30, 2001, SR141716; and Kirkham and Williams, 2001, *Nutrition Research Reviews* 14: 65-86 show the use of;
3) CB1 receptor antagonists to enhance cognition: United States patent 5,596,106 to Cullinan et al., (Jan. 21, 1997) shows the use of(see column 2, lines 35-53 and column 24, lines 10-18);
4) CB1 agonists as analgesics to alleviate pain: United States patent 6,348,498 to Calignano et al., (Feb. 19, 2002) (see column 1, lines 65-67 and column 2, lines 1-3, which also refers to appetite control); United States patent 6,949,582 to Wallace (Sept. 27, 2005) also shows that cannabinoids are known analgesics (see column 4, lines 11-16);
5) Treatment of multiple sclerosis: United States patent 6,900,236 to Makriyannis et al. (May 31, 2005) (see column 13, lines 42-55, which also describes the treatment of pain and other conditions); and Pertwee 2002 *Pharmacology & Therapeutics* 95: 165-174 further demonstrates the link between cannabinoids and the treatment of multiple sclerosis;
6) Treatment of epilepsy: Wallace et al., 2001 *European Journal of Pharmacology* 428: 51-57; Wallace et al., 2003 *Journal of Pharmacology and Experimental Therapeutics* 307:129-137; and a commentary by Robyn Wallace in Current Literature (pages 93-95) all document the relationship between cannabinoids and control of epileptic seizures or convulsions.

7) United States patent 6,645,985 to Barth et al. (Nov. 11, 2003) confirms that several known uses for cannabinoid compounds which bind to CB1 receptors, e.g. craving for addictive drugs, memory and cognitive disorders, etc. (see column 8, line 32-67 and column 9, line 1-7).

Applicant submits that one of skill in the art would recognize that the cannabinoid agonists and silent antagonists of the present invention, which bind to CB1 receptors with physiological relevant affinities, would be useful for the treatment of such conditions. Therefore, Applicant submits that in addition to being enabled for the treatment of vomiting, nausea, and appetite loss, the claimed subject matter is also enabled for the treatment of such conditions or disorders.

In addition, claim 7 has now been amended to recite the conditions which can be treated by the compounds of the invention arranged according to which conditions may be treated by *agonists*, and which can be treated by *silent antagonists*. Support for this arrangement is found in the specification as filed. For example, uses of *agonists* (e.g. as analgesics, appetite stimulants, anticonvulsants, to treat multiple sclerosis, to treat nausea and vomiting, to treat epilepsy) are described in the paragraph beginning at line 8 on page 12 and line 7 of page 14, and conditions to be treated by *silent antagonists* (cognitive enhancers, appetite suppressants, to treat alcohol, tobacco, cocaine and marijuana dependence) are described in the following paragraph (lines 14-17 on page 12 and lines 15-23 on page 14).

Claim 8 has been cancelled, its subject matter having been incorporated into claim 7.

While reviewing the claims and application an error in claims 10 and 13 was noted. Those claims previously recited the treatment of pain and nausea, respectively, by a "silent antagonist". As can be seen in the description on pages 12 and 14 referred to above, CB1 agonists (not silent antagonists) would be used to treat those conditions. Claims 10 and 13 and their respective dependent claims have thus hereby been amended to recite the treatment of pain and nausea, respectively, by an agonist of the present invention, and in claims 12 and 15, the chemical representation of the silent antagonist O-2050 has been deleted (note brackets) and replaced by the agonists O-2113 and O-2048. Further, the formulas of claims 11 and 14 have been amended to include the proviso that if R is CH_3, then X must be CH_2 or a saturated C_2

carbon chain. If R is CH_3 and X is unsaturated, the resulting structure is a silent antagonist (e.g. see structure of O-2050 at the top of page 10). The specification clearly states that X may be either saturated or unsaturated (see, for example, page 8, line 17). By definition, an "agonist" thus requires elimination of X as unsaturated when R = CH_3.

The Examiner also inquires regarding what is meant by the term "silent antagonist that is a sulfonamide moiety". Firstly, Applicant notes that Examiner has not quoted the phrase correctly. The precise claim language is "silent antagonist that *includes* a sulfonamide moiety" (i.e. "includes", not "is"). Claims 9, 10, 13 and 16 recite: the compound (claim 9); its use in treating pain (claim 10); its use in treating nausea (claim 13); and its use to block the effects of a CB1 cannabinoid receptor agonist (claim 16). The first portion of the phrase, "silent antagonist", is clearly defined in the specification. A brief discussion is presented on page 1 at lines 15-22, and a detailed discussion of agonists, antagonists and silent antagonists is presented beginning on page 11 at line 6 and continuing on page 12 at lines 1-7. Silent antagonists are antagonists that bind to a receptor, thereby preventing an endogenous ligand from binding, but which do not produce any inverse agonist properties, i.e. they do not activate the receptor or produce an observable effect upon binding, other than to block access to the receptor so that other ligands cannot bind. Thus, their effect is "silent". This is an advantage, since the side effects of some antagonists are not desirable, e.g. they may activate the receptor in a manner opposite to that of an endogenous ligand (page 12, lines 3-7). Data presented in Examples 4 and 6 of the application demonstrate that the compounds of the invention do not act as a typical inverse agonists, but rather as "silent antagonists". In a GTPγS binding assay described in Example 4, the compound O-2050 failed to increase GTPγS, although it was able to compete with a highly efficient known cannabinoid for binding to the CB1 receptor. Likewise, in the mouse vas deferens system of Example 6, O-2050 exhibited the ability to bind to CB1 but did not produce negative stimulation, consistent with a lack of inverse agonist properties. O-2050 thus fulfils the criteria for being a "silent antagonist".

Applicant submits that the second portion of the phrase, "includes a sulfonamide moiety", would be readily understood by one of skill in the art. Applicant herewith supplies two articles referenced in the application (page 20, lines 15-17, Matassa et al., and Jacobs et al.) which

describe the incorporation of sulfonamide moieties into antagonists of peptidoleukotrienes. These articles were referred to by Examiner as not having been supplied. These articles are evidence of the state of the art at the time of filing of the present application. Applicant submits that, based on the explanations and data presented in the specification and the knowledge extant at the time the application was filed, one of skill in the art would fully understand the meaning of the phrase "silent antagonist that includes a sulfonamide moiety". Thus, claims 9, 10, 13 and 16 are fully enabled.

Since independent claims 9, 10, 13 and 16 are fully enabled, Applicant further submits that dependent claims 11 and 12 (dependent on claim 10) and 14 and 15 (dependent on claim 13) are also fully enabled. The chemical structures claimed for use in the dependent claims are fully described in the specification.

In view of the foregoing, Applicant respectfully requests reconsideration and withdrawal of this rejection.

New Claims

New claims 17-18 recite the treatment of obesity by use of a silent antagonist of a CB1 receptor. This use of the silent antagonists of the invention is described in the specification, for example, on page 14 at line15. The particular silent antagonist recited in new claim 18 is O-2050, the activity of which is described in the specification on page 22 in Table 2 and at lines 6-7, and in Examples 3-6. Thus, Applicant submits that these new claims are fully enabled by the specification. In addition, as described above, Applicant has herewith submitted two articles that indicate that CB1 antagonists are useful for the treatment of obesity.

New claims 19-20 recite the treatment of craving for drugs by use of a silent antagonist of a CB1 receptor. This use of the silent antagonists of the invention is described in the specification, for example, on page 12 at lines 15-16 and page 14, lines 18-19. Again, the particular silent antagonist recited in new claim 20 is O-2050, the activity of which is described in the specification on page 22 in Table 2 and at lines 6-7, and in Examples 3-6. Thus, Applicant submits that these new claims are fully enabled by the specification. In addition, as described above, Applicant has herewith submitted several articles that indicate that CB1 antagonists are useful for the treatment of drug craving.

Applicant respectfully requests examination and allowance of new claims 17-20.

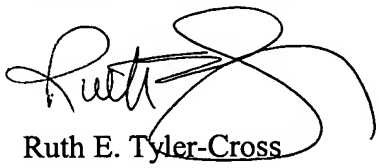
Conclusion

In view of the foregoing, it is requested that the application be reconsidered, that claims 1-7 and 9-20 be allowed, and that the application be passed to issue.

Should the Examiner find the application to be other than in condition for allowance, the Examiner is requested to contact the undersigned at 703-787-9400 (fax: 703-787-7557; email: ruth@wcc-ip.com) to discuss any other changes deemed necessary in a telephonic or personal interview.

If an extension of time is required for this response to be considered as being timely filed, a conditional petition is hereby made for such extension of time. Please charge any deficiencies in fees and credit any overpayment of fees to Attorney's Deposit Account No. 50-2041.

Respectfully submitted,



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Perspectives in Pharmacology

Cannabinoid CB_1 Receptor Antagonists as Promising New Medications for Drug Dependence

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ABSTRACT

This review examines the development of cannabinoid CB_1 receptor antagonists as a new class of therapeutic agents for drug addiction. Abused drugs [alcohol, opiates, Δ^9-tetrahydrocannabinol (Δ^9-THC), and psychostimulants, including nicotine] elicit a variety of chronically relapsing disorders by interacting with endogenous neural pathways in the brain. In particular, they share the common property of activating mesolimbic dopamine brain reward systems, and virtually all abused drugs elevate dopamine levels in the nucleus accumbens. Cannabinoid CB_1 receptors are expressed in this brain reward circuit and modulate the dopamine-releasing effects of Δ^9-THC and nicotine. Rimonabant (SR141716), a CB_1 receptor antagonist, blocks both the dopamine-releasing and discriminative and rewarding effects of Δ^9-THC in animals. Blockade of CB_1 receptor activity by genetic invalidation also decreases rewarding effects of opiates and alcohol in animals. Although CB_1 receptor blockade is generally ineffective in reducing the self-administration of cocaine in rodents and primates, it reduces the reinstatement of extinguished cocaine-seeking behavior produced by cocaine-associated conditioned stimuli and cocaine-priming injections. Likewise, CB_1 receptor blockade is effective in reducing nicotine-seeking behavior induced by re-exposure to nicotine-associated stimuli. Some of these findings have been recently validated in humans. In clinical trials, Rimonabant blocks the subjective effects of Δ^9-THC in humans and prevents relapse to smoking in exsmokers. Findings from both clinical and preclinical studies suggest that ligands blocking CB_1 receptors offer a novel approach for patients suffering from drug dependence that may be efficacious across different classes of abused drugs.

CB_1 Receptors Modulate the Brain Reward Pathway

Drug dependence is a chronic, relapsing disorder in which compulsive drug-seeking and -taking behavior persists despite serious negative consequences (American Psychiatric Association, 2000). Addictive substances, such as cannabinoids, opioids, ethanol, and psychostimulants, including nicotine, induce pleasant states or relieve distress, effects that contribute to their recreational use. After repeated exposure, adaptive changes occur in the central nervous system that lead to drug dependence (American Psychiatric Association, 2000). Although addictive drugs produce their effects through actions at various receptors in the brain, it is thought that their common effects on the activity of dopaminergic brain reward pathways is primarily responsible for their addictive properties (Koob, 1992a,b; Wise, 2004). Notably, the mesocorticolimbic system, which projects from the ventral tegmental area to the nucleus accumbens, cortical areas, and amygdala, is implicated in the rewarding effects of psychostimulants and other drugs of abuse, as well as the effects of nondrug natural rewards such as food (Wise, 1982). The involvement of dopamine in the rewarding effects of drugs of abuse is suggested by findings that most drugs abused by humans increase levels of dopamine in the nucleus

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ABBREVIATIONS: Δ^9-THC, Δ^9-tetrahydrocannabinol; SR141716, Rimonabant; CPP, conditioned place preference(s); FR, fixed ratio; HU-210, (6a*R*)-*trans*-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[*b*,*d*]pyran-9-methanol; AM-251, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide.

accumbens (Imperato et al., 1986; Pidoplichko et al., 1997) and that blockade of dopamine transmission reduces the rewarding effects of psychostimulants (Koob, 1992a,b); however, the role of dopamine seems more complex than simply mediating the primary reinforcing effects of drugs of abuse (Salamone et al., 2003; Wise, 2004). Recent evidence suggests that dopamine is strongly implicated in learning and conditioning processes (Schultz et al., 1997; Schultz, 2002) and in drug-seeking behavior (Phillips et al., 2003).

Marijuana is the most widely used illicit drug in the United States. The main psychoactive ingredient in marijuana is Δ^9-tetrahydrocannabinol (Δ^9-THC). Two forms of cannabinoid receptors, CB_1 and CB_2, have been cloned (Matsuda et al., 1990; Gerard et al., 1991; Munro et al., 1993). The CB_1 receptor and its splice variant, the CB_{1A} receptor, are predominantly found in the brain, with the highest density in the hippocampus, cerebellum, cortex, and striatum, whereas CB_2 receptors are located peripherally, principally associated with the immune system (Howlett et al., 2002). New data suggest the existence of an additional cannabinoid receptor (non-CB_1/non-CB_2) (see Wilson and Nicoll, 2002). Δ^9-THC may produce its effects by duplicating the effects of natural ligands for CB_1 receptors (anandamide, 2-arachidonylglycerol, and, perhaps, noladin ether), which have a shorter duration of action than synthetic or plant-derived cannabinoids and are implicated in various nervous system functions such as reward, memory, cognition, and pain perception (Wilson and Nicoll, 2002). Central nervous system effects produced by Δ^9-THC have been linked to the cannabinoid CB_1 receptor. As with other drugs of abuse, Δ^9-THC also produces an elevation in dopamine levels in the nucleus accumbens of rats (Chen et al., 1990) that is blocked by SR141716, a cannabinoid CB_1 receptor antagonist (Tanda and Di Chiara, 1997).

The potential utility of cannabinoid CB_1 receptor antagonists for the treatment of drug dependence has recently received considerable attention. This approach has been tested for Δ^9-THC and other types of drugs of abuse. This review focuses on the development of cannabinoid CB_1 receptor antagonists for the treatment of drug dependence. We will first summarize the main animal models used to assess subjective and rewarding/reinforcing effects of drugs of abuse and then summarize in Table 1 the preclinical and clinical findings related to CB_1 receptor blockade and the subjective and rewarding/reinforcing effects of different drugs of abuse in these models. The results obtained with various drugs of abuse will be presented by drug class. The putative neurobiological mechanisms underlying these effects will also be discussed. Although some drugs of abuse, such as ecstasy, are sometimes used together with marijuana (Croft et al., 2001), the involvement of cannabinoid mechanisms in the effects of these drugs has seldom been studied (Braida and Sala, 2002), and these limited findings will not be reviewed here.

Animal Models for Studying Effects of Drugs of Abuse

A variety of animal models are available to study the cardinal features of drug dependence (Schuster and Woods, 1968; Goldberg, 1975; Goldberg et al., 1975, 1979, 1981; Spealman and Goldberg, 1978; Katz and Goldberg, 1988; Markou et al., 1993; Everitt and Robbins, 2000; Schindler et al., 2002; Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004). The effects of CB_1 blockade have been evaluated using animals models for the subjective effects of drugs (drug discrimination), their rewarding/reinforcing properties [intravenous drug self-administration, conditioned place preference (CPP), and intracranial self-stimulation procedures], the influence of environmental factors on drug-seeking behavior (CPP, second-order schedules of drug self-administration, reinstatement of extinguished drug-seeking behavior, and other relapse models), and the withdrawal states associated with the abrupt termination of drug action (administration of selective antagonists after chronic exposure). We will mainly review results obtained with the drug discrimination procedure and the two most widely used procedures assessing rewarding or reinforcing effects of drugs in experimental animals: intravenous drug self-administration and drug-induced CPP procedures.

Drug Discrimination

Humans that abuse psychoactive drugs report characteristic subjective effects, and drug discrimination procedures in rats and monkeys are extensively used as animal models for subjective reports of drug effects in humans. The organism's ability to perceive and identify the characteristic interoceptive effects of drugs is thought to play a role in drug seeking, encouraging the development of this behavior and directing it toward one substance rather than another on the basis of relative potencies and effects (Stolerman and Shoaib, 1991). These interoceptive subjective effects of drugs are most frequently assessed in humans through the use of performance-assessment tasks and subject-rating scales. In animals, the interoceptive effects of drugs can serve as discriminative stimuli to indicate how to obtain a reinforcer such as a food pellet or how to avoid an electric shock. For this purpose, animals are trained under a discrete trial schedule of food pellet delivery or stimulus-shock termination to respond on one lever after an injection of a training dose of a drug and on the other lever after an injection of vehicle. Once animals learn to reliably make this discrimination, the subjective effects of different drugs can be compared, and the modulation of subjective effects of drugs of abuse by various pharmacological ligands can be measured.

Intravenous Drug Self-Administration

Natural rewards, such as water or food, and drugs of abuse may serve as positive reinforcers. For example, to assess the reinforcing effects of food, a food-deprived animal can be placed in a sound-attenuating chamber containing stimulus lights, response levers, and a device for dispensing food pellets automatically. Lever-pressing responses will occur with increasing frequency when they result in delivery of the food pellets, which, therefore, serve as positive reinforcers under these conditions. With intravenous drug self-administration procedures, a catheter implanted in a jugular vein allows the animal to intravenously self-administer a small amount of drug by pressing a lever. The administration of drug constitutes the event that positively reinforces the lever-pressing behavior, and reward is inferred if the frequency of responding subsequently increases (thus defining reinforcement). With these behavioral procedures, a stimulus light is often associated with delivery of the reinforcer. This stimulus, or cue, progressively gains motivational value by Pavlovian con-

TABLE 1
Main effect of CB_1 blockade on the subjective, discriminative, and rewarding/reinforcing effects of drugs of abuse in animal and human subjects

Species	Model	Results	Reference
Δ^9-THC			
Squirrel monkeys	i.v. self-administration under an FR10 schedule	SR141716 decreased self-administration	Tanda et al. (2000)
Rats and monkeys	Drug discrimination	SR141716 blocked discrimination of Δ^9-THC	Wiley et al. (1995); Jarbe et al. (2001); Solinas et al. (2003)
Humans	Reports of subjective drug effects	SR141716 blocked subjective effects of Δ^9-THC	Huestis et al. (2001)
Cocaine			
Squirrel monkeys	i.v. self-administration under an FR10 schedule	No effect of SR141716	Tanda et al. (2000)
Rats	Cocaine self-administration at a low ratio requirement (FR5)	No effect of SR141716	De Vries et al. (2001)
Rats	Relapse to drug-seeking behavior	SR141716 prevented relapse induced by cues and drug priming	De Vries et al. (2001)
Mice	Cocaine self-administration	No effect of CB_1 receptor invalidation	Cossu et al. (2001)
Mice	Cocaine-induced CPP	No effect of CB_1 receptor invalidation	Martin et al. (2000)
Rats	Cocaine-induced CPP	SR141716 blocked acquisition, but not expression, of CPP	Chaperon et al. (1998)
Opiates			
Rats	Heroin self-administration at high ratio requirements (FR10 and progressive ratio schedules)	SR141716 decreased self-administration	De Vries et al. (2003); Solinas et al. (2003)
Rats	Heroin self-administration at low ratio requirements (FR1–5)	No effect of SR141716	De Vries et al. (2003); Solinas et al. (2003)
Rats	Morphine-induced CPP	SR141716 blocked acquisition of CPP	Chaperon et al. (1998)
Mice	Morphine self-administration at a low ratio requirement (FR1)	CB_1 receptor invalidation blocked opiate self-administration	Cossu et al. (2001)
Mice	Morphine-induced CPP	CB_1 receptor invalidation blocked acquisition of CPP	Martin et al. (2000)
Alcohol			
Rats and mice	Oral ethanol intake and ethanol preference	SR141716 decreased oral ethanol intake	Arnone et al. (1997); Colombo et al. (1998); Rodriguez de Fonseca et al. (1999); Rinaldi-Carmona et al. (2004)
Mice	Oral ethanol intake	CB_1 receptor invalidation decreased ethanol intake and the effects of SR141716	Hungund et al. (2003); Poncelet et al. (2003); Naassila et al. (2004)
Mice	Acquisition of ethanol-induced CPP	CB_1 receptor invalidation reduced ethanol CPP	Houchi et al. (2004)
Nicotine			
Humans	Smoking cessation trial	SR141716 increased smoking cessation rates	Anthenelli and Despres (2004)
Rats	Self-administration of i.v. nicotine at a low ratio requirement (FR4)	SR141716 decreased self administration	Cohen et al. (2002)
Rats	Expression of CPP (stimulus-controlled behavior)	SR141716 blocked preferences for nicotine-paired environment	Le Foll and Goldberg (2004a)
Rats	Nicotine discrimination	No effect of SR141716	Cohen et al. (2002); Le Foll and Goldberg (2004a)
Mice	Nicotine-induced CPP	No CPP for nicotine in CB_1-deficient mice	Castane et al. (2002)
Mice	Self-administration of i.v. nicotine at a low ratio requirement (FR1)	No effect of CB_1 receptor invalidation	Cossu et al. (2001)

ditioning and can induce and maintain drug-seeking behavior and also reinstate drug-seeking behavior after extinction (Goldberg, 1975; Goldberg et al., 1975, 1983; de Wit and Stewart, 1981; Stewart, 1983; Self and Nestler, 1988; Meil and See, 1996; Arroyo et al., 1999), providing useful measures of the motivational effects of drug-related stimuli. Various schedules of reinforcement of drug self-administration behavior have been developed.

Under a fixed ratio (FR) schedule of intravenous drug injection, a fixed number of lever presses is necessary to obtain each injection of drug (e.g., one lever press for a fixed ratio 1, i.e., FR1, schedule). In contrast, under a progressive ratio schedule, the number of lever-press responses necessary to obtain a drug injection increases after each drug injection (Hodos, 1961). Thus, the number of responses the subject must make for each successive drug injection (the ratio value) is increased progressively until the subject fails to emit the required number of responses; this highest ratio (the "breaking point") is thought to reflect the reinforcing effectiveness of the drug. Self-administration studies have repeatedly shown that most drugs considered to be addictive in humans can serve as positive reinforcers for laboratory rats and monkeys, whereas nonaddictive drugs have given negative results in most cases (Katz and Goldberg, 1988; Balster, 1992). Once an animal has been trained to self-administer the drug, the influences of drug priming, stressors, or presentation of drug-associated cues on drug self-administration behavior or relapse to extinguished drug-seeking behavior provide useful measures for studying drug taking or relapse (Shalev et al., 2002).

Drug-Induced Conditioned Place Preferences

Another experimental animal model for exploring the rewarding effects of drugs of abuse is the CPP procedure. A distinctive environment (e.g., one compartment of a two- or three-compartment apparatus) is paired repeatedly with the administration of a drug, and a different environment is repeatedly associated with the administration of vehicle. CPP occurs when repeated administration of a drug in this particular environment results in the ability of that environment to elicit approach behavior and increased time contact (place preference) in the absence of the previously administered drug. It has been argued that CPP, like drug self-administration and a number of related phenomena, is an example of dopamine-mediated incentive learning and that

the approach behavior and increased time spent by animals in a drug-paired environment can be considered a measure of drug-seeking behavior (Bardo and Bevins, 2000; Le Foll and Goldberg, 2004a). CPP have been demonstrated for most drugs of abuse, as well as natural rewards such as food. The acquisition of a drug-induced CPP is likely to reflect the rewarding properties of a drug of abuse, whereas its expression reflects the influence on behavior of environmental stimuli previously associated with a drug's effects.

Effects of CB_1 Blockade on Effects of Drugs of Abuse

Δ^9-Tetrahydrocannabinol

Since the development of a rodent model of Δ^9-THC self-administration has so far been unsuccessful (Tanda and Goldberg, 2003), the drug discrimination model has been widely used to study cannabinoid effects in animals. Animals can learn to reliably discriminate Δ^9-THC from vehicle, and the cannabinoid CB_1 antagonist SR141716 produces reversible, dose-dependent antagonism of the discriminative stimulus effects of Δ^9-THC in rats (Wiley et al., 1995; Jarbe et al., 2001; Solinas et al., 2003) and monkeys (Wiley et al., 1995). When SR141716 was administered alone, it did not substitute for Δ^9-THC in rats (Wiley et al., 1995). Moreover, in humans, SR141716 (Rimonabant) was also able to block subjective effects induced by Δ^9-THC (Huestis et al., 2001). This selective cannabinoid antagonist also precipitated a withdrawal syndrome in cannabinoid-dependent animals (Tanda et al., 1999; Maldonado and Rodriguez de Fonseca, 2002). The precipitation of a physical withdrawal syndrome by SR141716 was associated with a reduction of dopamine levels in the shell of the nucleus accumbens in cannabinoid-dependent rats, but no such effects were found after the administration of SR141716 to saline-control rats (Tanda et al., 1999). Recently, a squirrel monkey model of Δ^9-THC intravenous self-administration has been developed (Tanda et al., 2000; Justinova et al., 2003). SR141716 almost entirely blocked the self-administration of Δ^9-THC in squirrel monkeys under an FR10 schedule of reinforcement (Tanda et al., 2000). These results suggest that blockade of cannabinoid CB_1 receptors may block both the subjective and rewarding effects of Δ^9-THC in humans.

Opiates

Functional interactions between cannabinoid and opioid neurotransmitter systems that are implicated in drug reinforcement/reward processes (Navarro et al., 2001; De Vries et al., 2003; Solinas et al., 2003) have been described previously (Manzanares et al., 1999). Notably, the discriminative (Solinas et al., 2004) and rewarding/reinforcing (Chen et al., 1990; Justinova et al., 2004) effects of Δ^9-THC are reversed by treatment with the opioid receptor antagonists naloxone and naltrexone. Selective μ-opioid receptor invalidation in mice also reduced the rewarding effects of Δ^9-THC, as assessed by the conditioned place preference procedure (Ghozland et al., 2002). These effects seem specific to the rewarding/reinforcing effects of Δ^9-THC, since naltrexone, an opiate antagonist, did not block the subjective effects of Δ^9-THC administration in humans (Wachtel and de Wit, 2000; Haney et al., 2003). Conversely, several studies have evaluated cannabinoid system modulation of the reinforcing effects of opiates. SR141716 treatment prevented the development of morphine-induced CPP (Chaperon et al., 1998), and cannabinoid CB_1 receptor knockout mice did not self-administer morphine (Cossu et al., 2001) or develop morphine-induced CPP (Martin et al., 2000). In agreement, blockade of cannabinoid CB_1 receptors by SR141716 markedly reduced responding for intravenous heroin injections under an FR5 schedule of reinforcement and to a greater extent under a progressive ratio schedule of reinforcement in rats (De Vries et al., 2003; Solinas et al., 2003). The cannabinoid CB_1 receptor agonist HU-210 reinstated heroin-seeking behavior following a 2-week extinction period, whereas SR141716 dose-dependently attenuated heroin seeking produced by a priming injection of heroin or re-exposure to heroin-associated stimuli (De Vries et al., 2003). Although SR141716 markedly decreased responding for heroin by rats under a progressive ratio schedule across a wide range of heroin doses, it had little effect on responding for food under a similar progressive ratio schedule (Solinas et al., 2003). In contrast to effects under the progressive ratio schedule, when responding was continuously reinforced under an FR1 schedule, SR141716 only reduced responding for low 12.5- and 25-μg/kg injection doses of heroin. The fact that heroin self-administration was affected in a different manner under these schedules is consistent with a behavioral economic analysis (Bickel et al., 2000), where the price of drug is considered to be the amount of effort (ratio size) required to obtain a fixed amount of drug. Thus, the effects of SR141716 on drug self-administration were more pronounced under a progressive ratio schedule of reinforcement (high price of drug), weaker under an FR5 schedule of self-administration (lower price of drug), and null under an FR1 schedule of self-administration of heroin or cocaine injections (very low price of drug). The effectiveness of cannabinoid CB_1 receptor blockade seems to depend on the price of the drug, with self-administration at high drug prices being notably sensitive to disruption. It is interesting to note that SR141716 did not modify the dopamine-releasing effect of heroin in the nucleus accumbens (Tanda and Di Chiara, 1997; Caille and Parsons, 2003).

Psychostimulants (Cocaine-Amphetamine)

Several experiments do not support, at first sight, an involvement of cannabinoid systems in the reinforcing effects of psychostimulants. CB_1 receptor-deficient mice learned to self-administer cocaine and amphetamine, as did their wild-type littermate controls (Cossu et al., 2001). Moreover, SR141716 administration did not interfere with cocaine self-administration in rats (De Vries et al., 2001) or monkeys (Tanda et al., 2000) trained under fixed ratio schedules of reinforcement (Fig. 1). This lack of effect of SR141716 did not reflect an insufficient dosage, since the doses of SR141716 tested were able to dramatically reduce Δ^9-THC self-administration in monkeys (Tanda et al., 2000) (Fig. 1). In contrast, AM-251, another CB_1 receptor antagonist, decreased the frequency of methamphetamine self-administration under a fixed ratio schedule in rats (decreased drug intake), whereas anandamide and *R*-methanandamide, two cannabinoid receptor agonists, tended to increase the frequency of methamphetamine self-administration (Vinklerova et al., 2002). SR141716 was also effective in blocking the acquisition, but not the expression, of cocaine-induced CPP (Chaperon et al., 1998). However, CB_1 receptor invalidation did not prevent the development of cocaine-induced CPP (Martin et al., 2000). These studies suggest a weak modulatory role of en-

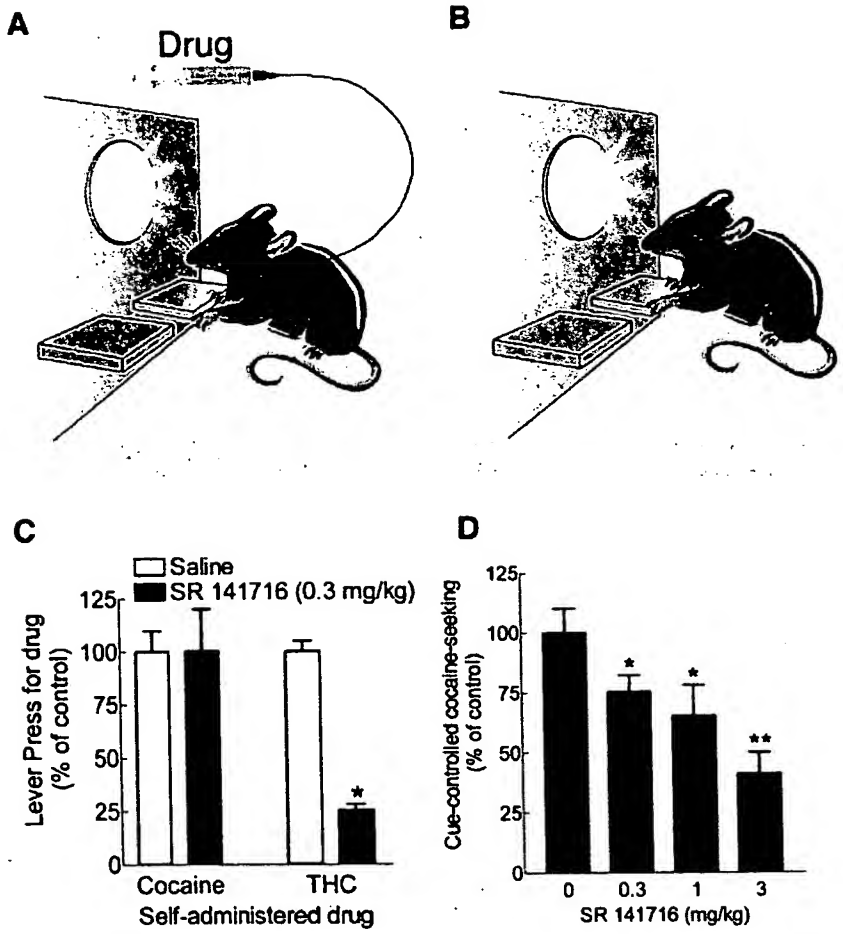

Fig. 1. SR141716 affects relapse in intravenous drug-self-administration studies. A, during repeated sessions, animals learned to press a lever to obtain intravenous injections of drug, and a brief light stimulus was associated with each drug injection. Through Pavlovian conditioning processes, this light stimulus progressively gained motivational properties. B, presentations of the brief light stimulus subsequently maintained drug-seeking behavior, even without drug delivery. C, SR141716 administration decreased Δ^9-THC self-administration, but not cocaine self-administration, in monkeys trained under an FR10 schedule of intravenous drug injection. Adapted from Tanda et al. (2000). D, SR141716 also did not affect cocaine self-administration in rats (data not shown), but it dose-dependently reduced relapse to cocaine-seeking behavior produced by cocaine-associated stimuli ("cues"). Adapted from De Vries et al. (2001).

docannabinoids on intake and, perhaps, on the rewarding/reinforcing effects of psychostimulants. The influence of the cannabinoid system on relapse has been demonstrated more clearly (De Vries et al., 2001). SR141716 reduced relapse to cocaine-seeking behavior produced by cocaine-paired stimuli (cues) (De Vries et al., 2001) (Fig. 1), whereas HU-210, a CB_1 receptor agonist, precipitated relapse to cocaine-seeking behavior (De Vries et al., 2001). Blockade of CB_1 receptors by SR141716 also was able to block relapse to cocaine-seeking behavior produced by a priming injection of cocaine but not by environmental stressors (De Vries et al., 2001). Likewise, SR141716 blocked the reinstatement of methamphetamine-seeking behavior in rats (Anggadiredja et al., 2004). Further experiments are needed to clarify the involvement of endogenous cannabinoid systems in the rewarding/reinforcing effects of psychostimulants.

Ethanol

Although the sites of actions for ethanol's effects in the brain are poorly understood, ethanol's reinforcing effects seem to involve dopamine pathways (Tabakoff and Hoffman, 1996). Recent evidence suggests that some of the pharmacological and behavioral effects of ethanol may also be mediated by endocannabinoid systems (Hungund et al., 2002). The expression of cannabinoid CB_1 receptors and their coupling to G proteins, as shown by the guanosine 5′-*O*-(3-[^{35}S]thio)triphosphate binding assay, seems to be different between alcohol-preferring and -avoiding mice (Hungund and Basavarajappa, 2000; Basavarajappa and Hungund, 2001). The pharmacological results obtained with SR141716 have been more pronounced with ethanol than with opiates and psychostimulants. Blockade of cannabinoid CB_1 receptors reduced alcohol intake (Arnone et al., 1997; Colombo et al., 1998; Rodriguez de Fonseca et al., 1999; Rinaldi-Carmona et al., 2004). The oral consumption of beer by rats, as assessed by a lick-based progressive ratio procedure, was decreased by CB_1 receptor blockade and increased by CB_1 receptor stimulation (Gallate and McGregor, 1999; Gallate et al., 1999, 2004). These effects have been reproduced in mice (Poncelet et al., 2003). The involvement of cannabinoid CB_1 receptors in the reinforcing/rewarding effects of ethanol is further indicated by findings that ethanol consumption is reduced in CB_1 receptor-deficient mice (Hungund et al., 2003; Poncelet et al., 2003; Naassila et al., 2004) and that the effects of SR141716 are abolished in these CB_1 receptor-deficient mice (Poncelet et al., 2003). Moreover, CB_1 receptor invalidation reduces ethanol-induced CPP (Houchi et al., 2004). All of these converging findings suggest that cannabinoid CB_1 receptor blockade may be an effective approach to the treatment of alcohol dependence in humans.

Nicotine

Nicotine and Δ^9-THC (in the form of marijuana) are often used in combination by humans. Several interactions have been described between nicotine and Δ^9-THC in animals (Valjent et al., 2002). Notably, the rewarding effects of these

two drugs measured by the CPP paradigm were additive when administered together; subthreshold doses of nicotine and Δ^9-THC, which were ineffective in inducing CPP by themselves, induced significant CPP when given together (Valjent et al., 2002). Interestingly, the cannabinoid CB_1 receptor antagonist SR141716 decreased nicotine self-administration in rats (Cohen et al., 2002), and nicotine was not able to induce conditioned place preferences in CB_1 receptor-deficient mice compared with their wild-type littermates (Castane et al., 2002). In contrast, CB_1 receptor knockout mice did seem to learn to self-administer nicotine (Cossu et al., 2001), suggesting that some of the actions of nicotine are not affected by cannabinoid CB_1 receptor blockade. Blockade of CB_1 receptors by SR141716 also did not block the discriminative stimulus effects of a high 0.4-mg/kg training dose of nicotine in one study (Cohen et al., 2002) and failed to change the discriminative stimulus effects of doses of nicotine ranging from 0.01 to 0.6 mg/kg in another study (Le Foll and Goldberg, 2004b). Interestingly, SR141716 dose-dependently blocked the dopamine-releasing effects of nicotine in the nucleus accumbens (Cohen et al., 2002) and the dopaminergic component of the nicotine discrimination (Cohen et al., 2002). Since dopamine release in the nucleus accumbens is thought to play a major role in the positive reinforcing effects of nicotine, these findings support a role for cannabinoid CB_1 receptors in modulating the rewarding/reinforcing effects of nicotine.

The maintenance of nicotine self-administration behavior in rats and monkeys often seems to critically depend on associated environmental stimuli (Goldberg et al., 1981; Caggiula et al., 2001; Cohen et al., 2004), and persistent effects of conditioned environmental stimuli previously associated with the effects of nicotine in tobacco may be a major determinant of relapse to smoking behavior in exsmokers. Acute administration of SR141716 blocks the expression of nicotine-induced conditioned place preferences in rats (Le Foll and Goldberg, 2004b) (see Fig. 2) and the influence of environmental stimuli on nicotine-seeking behavior (Cohen et al., 2004). These findings suggest that cannabinoid CB_1 receptor blockade reduced the effectiveness of conditioned motivational stimuli associated with nicotine injection. In agreement with this hypothesis, SR141716 administration has been shown to reduce intravenous nicotine self-administration behavior in rats (Cohen et al., 2002).

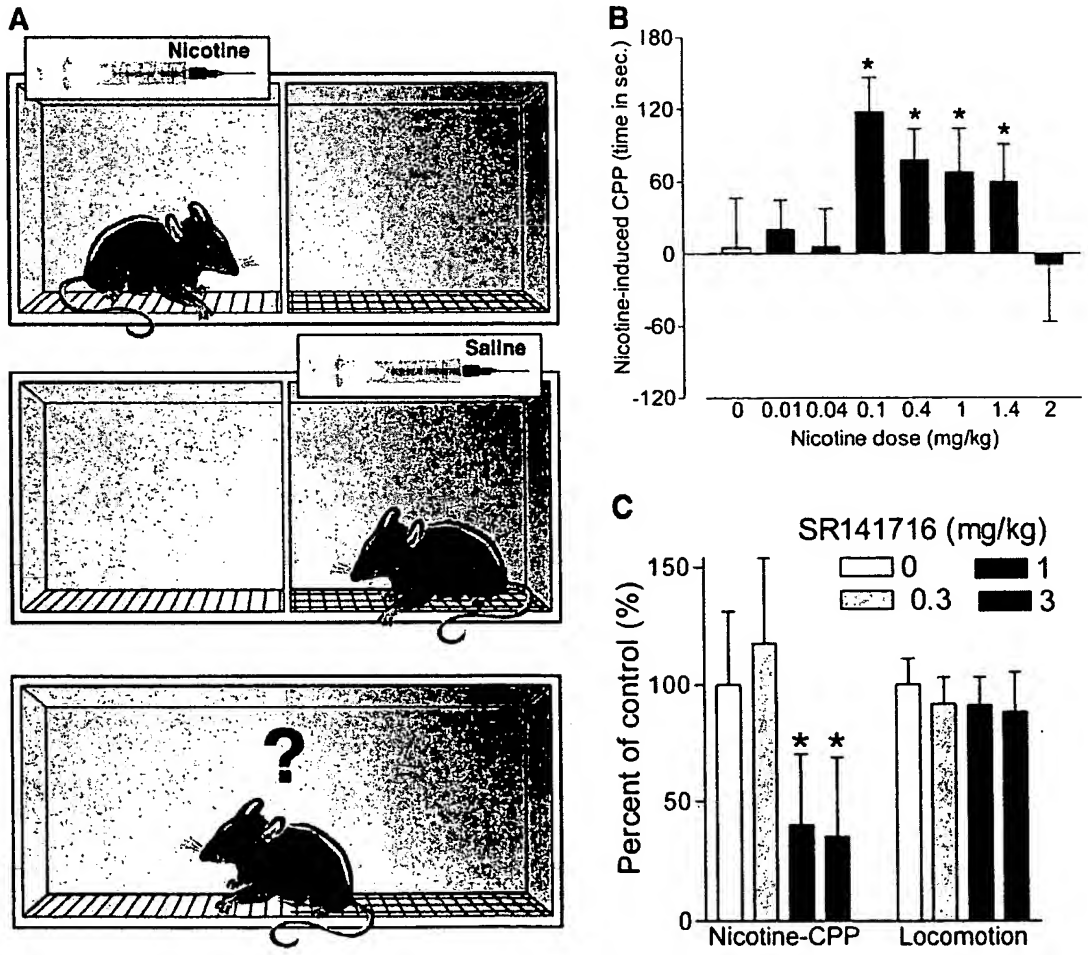

Fig. 2. SR141716 administration blocks nicotine-induced CPP. A, to induce CPP, a box with two discrete chambers, or environments, is used. Rats are repeatedly injected with nicotine before being placed in one environment and with saline before being placed in the other environment. Then, in a nicotine-free state, the animals are allowed access to both environments, and the amount of time spent in each environment is recorded. Adapted from Cami and Farre (2003) B, nicotine is able to induce significant conditioned place preferences over a large range of doses in rats. Results are expressed as the difference in time in seconds spent in the drug-paired side between the post- and preconditioning session. *, $P < 0.05$. Adapted from Le Foll and Goldberg (2004a). C, when SR141716 was administered acutely before the test session, it blocked the nicotine-induced conditioned place preference without interfering with the rat's locomotor activity. From Le Foll and Goldberg (2004b).

In human smokers, preliminary data from the STRATUS-US trial (smoking cessation in smokers motivated to quit) on the effects of SR141716 are promising (Anthenelli and Despres, 2004). This clinical study enrolled 787 smokers in 11 clinical trial sites in the United States. The participants were randomized to Rimonabant at a dose of 5 mg (n = 262) or 20 mg (n = 261) or a placebo. The study lasted 10 weeks, and the smokers were permitted to smoke during the first 2 weeks but were asked to abstain from smoking after this period. The quit rates for subjects in the 20-mg Rimonabant group were double that of the placebo group, and they showed a marked reduction in weight gain over the 10-week treatment (Anthenelli and Despres, 2004).

Neurobiological Pathways Affected by CB_1 Blockade

The mechanisms underlying the effects of CB_1 blockade on drug-induced reinforcement/reward and relapse to drug-seeking behavior remain unknown. Interestingly, SR141716 has been reported to block dopamine elevations in the nucleus accumbens produced by nicotine (Cohen et al., 2002) and Δ^9-THC (Tanda et al., 1997), and SR141716 is effective in decreasing the intravenous self-administration of these two drugs (Tanda et al., 1997; Cohen et al., 2002). In contrast, SR141716 is ineffective in blocking the dopamine-releasing effect of opiates in the nucleus accumbens (Tanda and Di Chiara, 1997) and is also ineffective in blocking opiate self-administration when the opiate is continuously available under an FR1 schedule of reinforcement (De Vries et al., 2003; Solinas et al., 2003). Further studies evaluating the effects of cannabinoid CB_1 receptor blockade on the dopamine-releasing effects of ethanol and cocaine are needed to confirm the putative relation between blockade of the dopamine-releasing effect of a drug in the nucleus accumbens and blockade of its reinforcing effects with self-administration procedures.

Environmental stimuli associated with drug self-administration can also produce dopamine elevations in the nucleus accumbens (Ito et al., 2000), and it is possible that SR141716 would also block such conditioned elevations in dopamine levels, which could result in a decreased efficacy of drug-paired stimuli and therefore reduce the tendency to relapse (De Vries et al., 2001, 2003; Cohen et al., 2004; Le Foll and Goldberg, 2004b). It is also likely that drug-priming effects that lead to relapse to drug-seeking behavior may be mediated through elevation of dopamine levels (Phillips et al., 2003). Further studies are needed to confirm the role of blockade of dopamine transmission in the behavioral effects of SR141716.

It is interesting to note that a profile similar to that described above with cannabinoid CB_1 receptor antagonists has been described with dopamine D_3 receptor ligands, which also reduce drug-seeking behavior induced by drug-associated stimuli (Pilla et al., 1999; Di Ciano et al., 2003) and block drug-induced conditioning processes (Le Foll et al., 2000, 2002, 2003a,b, 2004b; Vorel et al., 2002; Francès et al., 2004) but do not alter cocaine self-administration at a low fixed ratio value (Pilla et al., 1999). Some effects of SR141716 are diminished in dopamine D_3 receptor-deficient mice (Duarte et al., 2003), suggesting that dopamine D_3 receptors are involved in CB_1 receptor-mediated processes. Since dopamine D_3 receptors and cannabinoid CB_1 receptors are both expressed in the mesolimbic dopamine brain reward circuit (Mailleux and Vanderhaeghen, 1992; Diaz et al., 2000; Le Foll et al., 2002, 2003a,b), these two types of receptors may control the dopamine-releasing effect of drug-associated cues. These effects are probably mediated through the ventral tegmental area, the nucleus accumbens, or the amygdala (Le Foll et al., 2002, 2004a). An increase of monoaminergic neurotransmission in the medial prefrontal cortex may also be implicated in these behavioral effects (Lacroix et al., 2003; Tzavara et al., 2003).

Since cannabinoid CB_1 receptors are widely expressed throughout the brain, it seems likely that several different neurotransmitter systems are affected by cannabinoid CB_1 receptor blockade (Howlett et al., 2002). For example, CB_1 receptors are expressed in areas of the hypothalamus known to regulate appetite (Schwartz et al., 2000; Cota et al., 2003). Blockade of cannabinoid CB_1 receptors seems to decrease appetite and food intake, and CB_1 receptor antagonists are promising new medications for obesity (Black, 2004). Furthermore, blockade of cannabinoid CB_1 receptors by SR141716 prevents the development of food-induced CPP (Chaperon et al., 1998). Nevertheless, the neurobiological mechanisms underlying these effects are still unclear and may also involve dopaminergic transmission (Duarte et al., 2003). Further work is needed to determine whether similar or different neurotransmitter systems are involved in the effects of cannabinoid CB_1 receptor blockade on appetite and drug-seeking behavior.

Cannabinoid CB_1 Receptor Blockade: A Step Forward in Drug-Dependence Therapy?

Despite advances in the understanding of neurobiological and behavioral mechanisms that lead to drug dependence over the last 20 years, no effective treatment is yet available for cocaine or Δ^9-THC dependence. Moreover, medications available for ethanol, nicotine, or opioid dependence are ineffective in many subjects. For example, the rate of smoking cessation by subjects entering into clinical trials that combine effective medication and behavioral and cognitive therapy is around 30% at one year; most subjects relapse (Fiore, 2000). Cannabinoid CB_1 receptor antagonists represent a potentially useful tool not only for blocking the direct reinforcing effects of Δ^9-THC, nicotine, and ethanol, but also for preventing relapse to the use of various drugs of abuse, including cocaine, methamphetamine, and heroin. In addition, environmental stimuli seem to be one of the major factors that can trigger relapse to drug use in abstinent drug abusers. This process is not only critical for psychostimulant abuse, but also for nicotine and heroin abuse (Wikler, 1973; Childress et al., 1992; O'Brien et al., 1992, 1998), and probably for other drugs of abuse such as ethanol. By reducing the motivational effects of drug-related environmental stimuli, cannabinoid CB_1 receptor antagonists might, therefore, provide an effective means for preventing relapse to drug-seeking behavior in abstinent drug abusers, providing a promising new tool for the treatment of dependence on a wide range of abused drugs.

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References

American Psychiatric Association (2000) *Diagnostic and Statistical Manual of Mental Disorders*, 4th ed, American Psychiatric Association, Washington DC.

Anggadiredja K, Nakamichi M, Hiranita T, Tanaka H, Shoyama Y, Watanabe S, and Yamamoto T (2004) Endocannabinoid system modulates relapse to methamphetamine seeking: possible mediation by the arachidonic acid cascade. *Neuropsychopharmacology* 29:1470–1478.

Anthenelli RM and Despres JP (2004) Effects of Rimonabant in the reduction of major cardiovascular risk factors. Results from the STRATUS-US trial (smoking cessation in smokers motivated to quit), in *American College of Cardiology 53rd Annual Scientific Session*; 2004 Mar 7–10; New Orleans, LA.

Arnone M, Maruani J, Chaperon F, Thiebot MH, Poncelet M, Soubrie P, and Le Fur G (1997) Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology (Berl)* 132:104–106.

Arroyo M, Markou A, Robbins TW, and Everitt BJ (1999) Acquisition, maintenance and reinstatement of intravenous cocaine self-administration under a second-order schedule of reinforcement in rats: effects of conditioned cues and continuous acces to cocaine. *Psychopharmacology* 140:331–344.

Balster RL (1992) Preclinical methods for the development of pharmacotherapies of cocaine abuse, in *Problems of Drug Dependence* (Harris LS ed) pp 160–164, National Institute on Drug Abuse, Rockville, MD.

Bardo MT and Bevins RA (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl)* 153:31–43.

Basavarajappa BS and Hungund BL (2001) Cannabinoid receptor agonist-stimulated [35S]guanosine triphosphate gammaS binding in the brain of C57BL/6 and DBA/2 mice. *J Neurosci Res* 64:429–436.

Bickel WK, Marsch LA, and Carroll ME (2000) Deconstructing relative reinforcing efficacy and situating the measures of pharmacological reinforcement with behavioral economics: a theoretical proposal. *Psychopharmacology (Berl)* 153:44–56.

Black SC (2004) Cannabinoid receptor antagonists and obesity. *Curr Opin Investig Drugs* 5:389–394.

Braida D and Sala M (2002) Role of the endocannabinoid system in MDMA intracerebral self-administration in rats. *Br J Pharmacol* 136:1089–1092.

Caggiula AR, Donny EC, White AR, Chaudhri N, Booth S, Gharib MA, Hoffman A, Perkins KA, and Sved AF (2001) Cue dependency of nicotine self-administration and smoking. *Pharmacol Biochem Behav* 70:515–530.

Caille S and Parsons LH (2003) SR141716A reduces the reinforcing properties of heroin but not heroin-induced increases in nucleus accumbens dopamine in rats. *Eur J Neurosci* 18:3145–3149.

Cami J and Farre M (2003) Drug addiction. *N Engl J Med* 349:975–986.

Chaperon F, Soubrie P, Puech AJ, and Thiebot MH (1998) Involvement of central cannabinoid (CB1) receptors in the establishment of place conditioning in rats. *Psychopharmacology (Berl)* 135:324–332.

Chen JP, Paredes W, Li J, Smith D, Lowinson J, and Gardner EL (1990) Delta 9-tetrahydrocannabinol produces naloxone-blockable enhancement of presynaptic basal dopamine efflux in nucleus accumbens of conscious, freely-moving rats as measured by intracerebral microdialysis. *Psychopharmacology (Berl)* 102:156–162.

Childress E, R., Roohsenow DJ, Robbins SH and O'Brien CP (1992) Classically conditioned factors in drug dependence, in *Substance Abuse: A Comprehensive Textbook* (Lowinson W, Luiz P, Millman RB, and Langard JG eds) pp 56–69, Williams and Wilkins, Baltimore.

Cohen C, Perrault G, Griebel G, and Soubrie P (2004) Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal: reversal by the cannabinoid (CB(1)) receptor antagonist, Rimonabant (SR141716). *Neuropsychopharmacology*, in press.

Cohen C, Perrault G, Voltz C, Steinberg R, and Soubrie P (2002) SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. *Behav Pharmacol* 13:451–463.

Colombo G, Agabio R, Fa M, Guano L, Lobina C, Loche A, Reali R, and Gessa GL (1998) Reduction of voluntary ethanol intake in ethanol-preferring sP rats by the cannabinoid antagonist SR-141716. *Alcohol Alcohol* 33:126–130.

Cossu G, Ledent C, Fattore L, Imperato A, Bohme GA, Parmentier M, and Fratta W (2001) Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. *Behav Brain Res* 118:61–65.

Cota D, Marsicano G, Tschop M, Grubler Y, Flachskamm C, Schubert M, Auer D, Yassouridis A, Thone-Reineke C, Ortmann S, et al. (2003) The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Investig* 112:423–431.

Croft RJ, Mackay AJ, Mills AT, and Gruzelier JG (2001) The relative contributions of ecstasy and cannabis to cognitive impairment. *Psychopharmacology (Berl)* 153: 373–379.

Deroche-Gamonet V, Belin D, and Piazza PV (2004) Evidence for addiction-like behavior in the rat. *Science (Wash DC)* 305:1014–1017.

De Vries TJ, Homberg JR, Binnekade R, Raaso H, and Schoffelmeer AN (2003) Cannabinoid modulation of the reinforcing and motivational properties of heroin and heroin-associated cues in rats. *Psychopharmacology (Berl)* 168:164–169.

De Vries TJ, Shaham Y, Homberg JR, Crombag H, Schuurman K, Dieben J, Vanderschuren LJ, and Schoffelmeer AN (2001) A cannabinoid mechanism in relapse to cocaine seeking. *Nat Med* 7:1151–1154.

de Wit H and Stewart J (1981) Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology* 75:134–143.

Diaz J, Pilon C, Le Foll B, Gros C, Triller A, Schwartz JC, and Sokoloff P (2000) Dopamine D3 receptors expressed by all mesencephalic dopamine neurons. *J Neurosci* 20:8677–8684.

Di Ciano P, Underwood RJ, Hagan JJ, and Everitt BJ (2003) Attenuation of cue-controlled cocaine-seeking by a selective D3 dopamine receptor antagonist SB-277011-A. *Neuropsychopharmacology* 28:329–338.

Duarte C, Alonso R, Bichet N, Cohen C, Soubrie P, and Thiebot MH (2003) Blockade by the cannabinoid CB1 receptor antagonist, rimonabant (SR141716), of the potentiation by quinelorane of food-primed reinstatement of food-seeking behavior. *Neuropsychopharmacology* 29:911–920.

Everitt BJ and Robbins TW (2000) Second-order schedules of drug reinforcement in rats and monkeys: measurement of reinforcing efficacy and drug-seeking behaviour. *Psychopharmacology (Berl)* 153:17–30.

Fiore MC (2000) US public health service clinical practice guideline: treating tobacco use and dependence. *Respir Care* 45:1200–1262.

Francès H, Le Foll B, Diaz J, Smirnova M, and Sokoloff P (2004) Role of DRD3 in morphine-induced conditioned place preference using drd3-knockout mice. *Neuroreport* 15:2245–2249.

Gallate JE, Mallet PE, and McGregor IS (2004) Combined low dose treatment with opioid and cannabinoid receptor antagonists synergistically reduces the motivation to consume alcohol in rats. *Psychopharmacology (Berl)* 173:210–216.

Gallate JE and McGregor IS (1999) The motivation for beer in rats: effects of ritanserin, naloxone and SR 141716. *Psychopharmacology (Berl)* 142:302–308.

Gallate JE, Saharov T, Mallet PE, and McGregor IS (1999) Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. *Eur J Pharmacol* 370:233–240.

Gerard CM, Mollereau C, Vassart G, and Parmentier M (1991) Molecular cloning of a human cannabinoid receptor which is also expressed in testis. *Biochem J* 279 (Pt 1):129–134.

Ghozland S, Matthes HW, Simonin F, Filliol D, Kieffer BL, and Maldonado R (2002) Motivational effects of cannabinoids are mediated by mu-opioid and kappa-opioid receptors. *J Neurosci* 22:1146–1154.

Goldberg SR (1975) Stimuli associated with drug injections as events that control behavior. *Pharmacol Rev* 27:325–340.

Goldberg SR, Kelleher RT, and Goldberg DM (1981) Fixed-ratio responding under second-order schedules of food presentation or cocaine injection. *J Pharmacol Exp Ther* 218:271–281.

Goldberg SR, Kelleher RT, and Morse WH (1975) Second-order schedules of drug injection. *Fed Proc* 34:1771–1776.

Goldberg SR, Spealman RD, and Goldberg DM (1981) Persistent behavior at high rates maintained by intravenous self-administration of nicotine. *Science (Wash DC)* 214:573–575.

Goldberg SR, Spealman RD, and Kelleher RT (1979) Enhancement of drug-seeking behavior by environmental stimuli associated with cocaine or morphine injections. *Neuropharmacology* 18:1015–1017.

Goldberg SR, Spealman RD, Risner ME, and Henningfield JE (1983) Control of behavior by intravenous nicotine injections in laboratory animals. *Pharmacol Biochem Behav* 19:1011–1020.

Haney M, Bisaga A, and Foltin RW (2003) Interaction between naltrexone and oral THC in heavy marijuana smokers. *Psychopharmacology (Berl)* 166:77–85.

Hodos W (1961) Progressive ratio as a measure of reward strength. *Science (Wash DC)* 134:943–944.

Houchi H, Babovic D, Pierrefiche O, Ledent C, Daoust M, and Naassila M (2004) CB(1) receptor knockout mice display reduced ethanol-induced conditioned place preference and increased striatal dopamine D2 receptors. *Neuropsychopharmacology*, in press.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, et al. (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* 54:161–202.

Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET, and Frank RA (2001) Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. *Arch Gen Psychiatry* 58:322–328.

Hungund BL and Basavarajappa BS (2000) Distinct differences in the cannabinoid receptor binding in the brain of C57BL/6 and DBA/2 mice, selected for their differences in voluntary ethanol consumption. *J Neurosci Res* 60:122–128.

Hungund BL, Basavarajappa BS, Vadasz C, Kunos G, Rodriguez de Fonseca F, Colombo G, Serra S, Parsons L, and Koob GF (2002) Ethanol, endocannabinoids and the cannabinoidergic signaling system. *Alcohol Clin Exp Res* 26:565–574.

Hungund BL, Szakall I, Adam A, Basavarajappa BS, and Vadasz C (2003) Cannabinoid CB1 receptor knockout mice exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens. *J Neurochem* 84:698–704.

Imperato A, Mulas A, and Di Chiara G (1986) Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. *Eur J Pharmacol* 132:337–338.

Ito R, Dalley JW, Howes SR, Robbins TW, and Everitt BJ (2000) Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. *J Neurosci* 20:7489–7495.

Jarbe TU, Lamb RJ, Lin S, and Makriyannis A (2001) (R)-methanandamide and delta 9-THC as discriminative stimuli in rats: tests with the cannabinoid antagonist SR-141716 and the endogenous ligand anandamide. *Psychopharmacology (Berl)* 156:369–380.

Justinova Z, Tanda G, Munzar P, and Goldberg SR (2004) The opioid antagonist naltrexone reduces the reinforcing effects of delta 9 tetrahydrocannabinol (THC) in squirrel monkeys. *Psychopharmacology (Berl)* 173:186–194.

Justinova Z, Tanda G, Redhi GH, and Goldberg SR (2003) Self-administration of delta9-tetrahydrocannabinol (THC) by drug naive squirrel monkeys. *Psychopharmacology (Berl)* 169:135–140.

Katz JL and Goldberg SR (1988) Preclinical assessment of abuse liability of drugs. *Agents Actions* 23:18–26.

Koob GF (1992a) Dopamine, addiction and reward. *Semin Neurosci* 4:139–148.

Koob GF (1992b) Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol Sci* 13:177–184.

Lacroix LP, Hows ME, Shah AJ, Hagan JJ, and Heidbreder CA (2003) Selective

antagonism at dopamine D3 receptors enhances monoaminergic and cholinergic neurotransmission in the rat anterior cingulate cortex. *Neuropsychopharmacology* 28:839–849.

Le Foll B, Diaz J, and Sokoloff P (2003a) Increased dopamine D3 receptor expression accompanying behavioural sensitization to nicotine in rats. *Synapse* 47:176–183.

Le Foll B, Diaz J, and Sokoloff P (2004a) Neuroadaptations to hyperdopaminergia in dopamine D3 receptor deficient mice. *Life Sci*, 1281–1296.

Le Foll B, Francès H, Diaz J, Schwartz J-C, and Sokoloff P (2002) Role of the dopamine D3 receptor in reactivity to cocaine-associated cues in mice. *Eur J Neurosci* 15:2016–2026.

Le Foll B and Goldberg SR (2004a) Nicotine induces conditioned place preferences over a large range of doses in rats. *Psychopharmacology (Berl)*, in press.

Le Foll B and Goldberg SR (2004b) Rimonabant, a CB_1 antagonist, blocks nicotine-conditioned place preferences. *Neuroreport* 15:2139–2143.

Le Foll B, Schwartz J-C, and Sokoloff P (2000) Dopamine D3 receptor agents as potential new medications for drug addiction. *Eur Psychiatry* 15:140–146.

Le Foll B, Schwartz J-C, and Sokoloff P (2003b) Disruption of nicotine conditioning by dopamine D3 receptor ligands. *Molecular Psychiatry* 8:225–230.

Le Foll B, Sokoloff P, Stark H, and Goldberg SR (2004b) Dopamine D3 ligands block nicotine-induced conditioned place preferences through a mechanism that does not involve discriminative-stimulus or antidepressant-like effects. *Neuropsychopharmacology*, in press.

Mailleux P and Vanderhaeghen JJ (1992) Distribution of neuronal cannabinoid receptor in the adult rat brain: a comparative receptor binding radioautography and in situ hybridization histochemistry. *Neuroscience* 48:655–668.

Maldonado R and Rodriguez de Fonseca F (2002) Cannabinoid addiction: behavioral models and neural correlates. *J Neurosci* 22:3326–3331.

Manzanares J, Corchero J, and Fuentes JA (1999) Opioid and cannabinoid receptor-mediated regulation of the increase in adrenocorticotropin hormone and corticosterone plasma concentrations induced by central administration of delta(9)-tetrahydrocannabinol in rats. *Brain Res* 839:173–179.

Markou A, Weiss F, Gold LH, Caine B, Schulteis G, and Koob GF (1993) Animal models of drug craving. *Psychopharmacology* 112:163–182.

Martin M, Ledent C, Parmentier M, Maldonado R, and Valverde O (2000) Cocaine, but not morphine, induces conditioned place preference and sensitization to locomotor responses in CB1 knockout mice. *Eur J Neurosci* 12:4038–4046.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, and Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature (Lond)* 346:561–564.

Meil WM and See RE (1996) Conditioned cue recovery of responding following prolonged withdrawal from self-administered cocaine in rats: an animal model of relapse. *Behav Pharmacol* 7:754–763.

Munro S, Thomas KL, and Abu-Shaar M (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature (Lond)* 365:61–65.

Naassila M, Pierrefiche O, Ledent C, and Daoust M (2004) Decreased alcohol self-administration and increased alcohol sensitivity and withdrawal in CB1 receptor knockout mice. *Neuropharmacology* 46:243–253.

Navarro M, Carrera MR, Fratta W, Valverde O, Cossu G, Fattore L, Chowen JA, Gomez R, del Arco I, Villanua MA, et al. (2001) Functional interaction between opioid and cannabinoid receptors in drug self-administration. *J Neurosci* 21:5344–5350.

O'Brien CP, Childress AR, Ehrman RA, and Robbins SJ (1998) Conditioning factors in drug abuse: can they explain compulsion. *J Psychopharmacol* 12:15–22.

O'Brien CP, Childress AR, McMellan AT, and Ehrman RA (1992) A learning model of addiction. *Res Publ Assoc Res Nerv Ment Dis* 70:157–177.

Phillips PE, Stuber GD, Heien ML, Wightman RM, and Carelli RM (2003) Subsecond dopamine release promotes cocaine seeking. *Nature (Lond)* 422:614–618.

Pidoplichko VI, DeBiasi M, Williams JT, and Dani JA (1997) Nicotine activates and desensitizes midbrain dopamine neurons. *Nature (Lond)* 390:401–404.

Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, Schwartz JC, Everitt BJ, and Sokoloff P (1999) Selective inhibition of cocaine-seeking behaviour by a partial dopamine D_3 receptor agonist. *Nature (Lond)* 400:371–375.

Poncelet M, Maruani J, Calassi R, and Soubrie P (2003) Overeating, alcohol and sucrose consumption decrease in CB1 receptor deleted mice. *Neurosci Lett* 343: 216–218.

Rinaldi-Carmona M, Barth F, Congy C, Martinez S, Oustric D, Perio A, Poncelet M, Maruani J, Arnone M, Finance O, et al. (2004) SR147778 [5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-ethyl-*N*-(1-piperidinyl)-1*H*-pyrazole-3-carboxamide], a new potent and selective antagonist of the CB1 cannabinoid receptor. Biochemical and pharmacological characterization. *J Pharmacol Exp Ther* 310:905–914.

Rodriguez de Fonseca F, Roberts AJ, Bilbao A, Koob GF, and Navarro M (1999) Cannabinoid receptor antagonist SR141716A decreases operant ethanol self administration in rats exposed to ethanol-vapor chambers. *Zhongguo Yao Li Xue Bao* 20:1109–1114.

Salamone JD, Correa M, Mingote S, and Weber SM (2003) Nucleus accumbens dopamine and the regulation of effort in food-seeking behavior: implications for studies of natural motivation, psychiatry and drug abuse. *J Pharmacol Exp Ther* 305:1–8.

Schindler CW, Panlilio LV, and Goldberg SR (2002) Second-order schedules of drug self-administration in animals. *Psychopharmacology (Berl)* 163:327–344.

Schultz W (2002) Getting formal with dopamine and reward. *Neuron* 36:241–263.

Schultz W, Dayan P, and Montague PR (1997) A neural substrate of prediction and reward. *Science (Wash DC)* 275:1593–1599.

Schuster CR and Woods JH (1968) The conditioned reinforcing effects of stimuli associated with morphine reinforcement. *Int J Addict* 3:223–230.

Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, and Baskin DG (2000) Central nervous system control of food intake. *Nature (Lond)* 404:661–671.

Self DW and Nestler EJ (1988) Relapse to drug-seeking: neural and molecular mechanisms. *Drug Alcohol Depend* 51:49–60.

Shalev U, Grimm JW, and Shaham Y (2002) Neurobiology of relapse to heroin and cocaine seeking: a review. *Pharmacol Rev* 54:1–42.

Solinas M, Panlilio LV, Antoniou K, Pappas LA, and Goldberg SR (2003) The cannabinoid CB1 antagonist *N*-piperidinyl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (SR-141716A) differentially alters the reinforcing effects of heroin under continuous reinforcement, fixed ratio, and progressive ratio schedules of drug self-administration in rats. *J Pharmacol Exp Ther* 306:93–102.

Solinas M, Zangen A, Thiriet N, and Goldberg SR (2004) Beta-endorphin elevations in the ventral tegmental area regulate the discriminative effects of delta-9-tetrahydrocannabinol. *Eur J Neurosci* 19:3183–3192.

Spealman RD and Goldberg SR (1978) Drug self-administration by laboratory animals: Control by schedules of reinforcement. *Annu Rev Pharmacol Toxicol* 18:313–339.

Stewart J (1983) Conditioned and unconditioned drug effects in relapse to opiate and stimulant drug-administration. *Prog Neuropsychopharmacol Biol Psychiatry* 7:591–597.

Stolerman IP and Shoaib M (1991) The neurobiology of tobacco addiction. *Trends Pharmacol Sci* 12:467–473.

Tabakoff B and Hoffman PL (1996) Alcohol addiction: an enigma among us. *Neuron* 16:909–912.

Tanda G and Di Chiara G (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common m1 opioid receptor mechanism. *Science (Wash DC)* 276:2048–2050.

Tanda G and Goldberg SR (2003) Cannabinoids: reward, dependence and underlying neurochemical mechanisms—a review of recent preclinical data. *Psychopharmacology (Berl)* 169:115–134.

Tanda G, Loddo P, and Di Chiara G (1999) Dependence of mesolimbic dopamine transmission on delta9-tetrahydrocannabinol. *Eur J Pharmacol* 376:23–26.

Tanda G, Munzar P, and Goldberg SR (2000) Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. *Nat Neurosci* 3:1073–1074.

Tanda G, Pontieri FE, and Di Chiara G (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. *Science (Wash DC)* 276:2048–2050.

Tzavara ET, Davis RJ, Perry KW, Li X, Salhoff C, Bymaster FP, Witkin JM, and Nomikos GG (2003) The CB1 receptor antagonist SR141716A selectively increases monoaminergic neurotransmission in the medial prefrontal cortex: implications for therapeutic actions. *Br J Pharmacol* 138:544–553.

Valjent E, Mitchell JM, Besson MJ, Caboche J, and Maldonado R (2002) Behavioural and biochemical evidence for interactions between delta 9-tetrahydrocannabinol and nicotine. *Br J Pharmacol* 135:564–578.

Vanderschuren LJ and Everitt BJ (2004) Drug seeking becomes compulsive after prolonged cocaine self-administration. *Science (Wash DC)* 305:1017–1019.

Vinklerova J, Novakova J, and Sulcova A (2002) Inhibition of methamphetamine self-administration in rats by cannabinoid receptor antagonist AM 251. *J Psychopharmacol* 16:139–143.

Vorel SR, Ashby CRJ, Paul M, Liu X, Hayes R, Hagan JJ, Middlemiss DN, Stemp G, and Gardner EL (2002) Dopamine D3 receptor antagonism inhibits cocaine-seeking and cocaine-enhanced brain reward in rats. *J Neurosci* 22:9595–9603.

Wachtel SR and de Wit H (2000) Naltrexone does not block the subjective effects of oral delta(9)-tetrahydrocannabinol in humans. *Drug Alcohol Depend* 59:251–260.

Wikler A (1973) Dynamics of drug dependence. *Arch Gen Psychiatry* 28:611–616.

Wiley JL, Lowe JA, Balster RL, and Martin BR (1995) Antagonism of the discriminative stimulus effects of delta 9-tetrahydrocannabinol in rats and rhesus monkeys. *J Pharmacol Exp Ther* 275:1–6.

Wilson RI and Nicoll RA (2002) Endocannabinoid signaling in the brain. *Science (Wash DC)* 296:678–682.

Wise RA (1982) Neuroleptics and operant behavior: the anhedonia hypothesis. *Behav Brain Res* 5:39–87.

Wise RA (2004) Dopamine, learning and motivation. *Nat Rev Neurosci* 5:483–494.

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PROGRAM AND ABSTRACTS

SR141716, A SELECTIVE ANTAGONIST OF CB_1 RECEPTORS AND OBESITY

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A commonly reported effect of marijuana intoxication is spontaneous compulsive eating. Moreover, the cannabinoid agonist Δ^9-THC, induces over-consumption in humans and animals and anandamide induces overeating in rodents. On the other hand, SR141716, a selective CB_1 receptor antagonist decreases voluntary food intake in food deprived and non - food deprived rats. It selectively reduces sucrose feeding and drinking as well as NPY - induced sucrose drinking in rats. SR141716 selectively reduces food intake to highly palatable sweet food without modification of the standard food intake in marmosets and finally it reduces the consumption of fat diet and significantly reduces weight gain in Zucker obese rats.

In humans, SR141716 was tested in obese male subjects (BMI >27) in a double blind cross over study (20 mg once a day versus placebo). The patients were treated for 7 days + 28 days wash out. SR141716 had no effect on taste and spit tests, yet induced a significant decrease of hunger (Visual analogue scale), caloric intake and weight. Moreover in a subsequent study, at doses of 5, 10 and 20 mg once daily, SR141716 was able to significantly reduce body weight in obese patients when compared to placebo. The observed decrease in weight did not reach a plateau during the 4 month duration of the study and the tolerability of SR141716 was excellent.

In conclusion, these observations provide strong support for a role of the cannabinoid system in the central regulation of feeding both in animals and humans. Moreover, SR141716 a selective antagonist of CB_1 receptors might be a useful drug for obesity treatment.

Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study

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Summary

Background In animal models, cannabinoid-1 receptor (CB_1) blockade produces a lean phenotype, with resistance to diet-induced obesity and associated dyslipidaemia. We assessed the effect of rimonabant, a selective CB_1 blocker, on bodyweight and cardiovascular risk factors in overweight or obese patients.

Methods 1507 patients with body-mass index 30 kg/m² or greater, or body-mass index greater than 27 kg/m² with treated or untreated dyslipidaemia, hypertension, or both, were randomised to receive double-blind treatment with placebo, 5 mg rimonabant, or 20 mg rimonabant once daily in addition to a mild hypocaloric diet (600 kcal/day deficit). The primary efficacy endpoint was weight change from baseline after 1 year of treatment in the intention-to-treat population.

Findings Weight loss at 1 year was significantly greater in patients treated with rimonabant 5 mg (mean −3·4 kg [SD 5·7]; p=0·002 *vs* placebo) and 20 mg (−6·6 kg [7·2]; p<0·001 vs placebo) compared with placebo (−1·8 kg [6·4]). Significantly more patients treated with rimonabant 20 mg than placebo achieved weight loss of 5% or greater (p<0·001) and 10% or greater (p<0·001). Rimonabant 20 mg produced significantly greater improvements than placebo in waist circumference, HDL-cholesterol, triglycerides, and insulin resistance, and prevalence of the metabolic syndrome. The effects of rimonabant 5 mg were of less clinical significance. Rimonabant was generally well tolerated with mild and transient side effects.

Interpretation CB_1 blockade with rimonabant 20 mg, combined with a hypocaloric diet over 1 year, promoted significant decrease of bodyweight and waist circumference, and improvement in cardiovascular risk factors.

Introduction

The prevalence of obesity continues to increase, with more than 50% of Europeans currently classified as overweight and up to 30% as clinically obese.[1,2] WHO has estimated that, yearly, about a quarter of a million deaths in Europe and more than 2·5 million deaths worldwide are weight-related, with cardiovascular disease as the leading cause.[3] Because few safe and effective drugs are available, the treatment of obesity remains one of the greatest unmet clinical needs of our time.

The newly discovered endocannabinoid system contributes to the physiological regulation of energy balance, food intake, and lipid and glucose metabolism through both central and peripheral effects.[4–6] This system consists of endogenous ligands and two types of G-protein-coupled cannabinoid receptors: CB_1, located in several brain areas and in a variety of peripheral tissues including adipose tissue, the gastrointestinal tract, the pituitary and adrenal glands, sympathetic ganglia, heart, lung, liver, and urinary bladder;[7,8] and CB_2, in the immune system.[9] The endocannabinoid system is overactivated in genetic animal models of obesity[5] and in response to exogenous stimuli such as excessive food intake.[10] Preclinical studies implicate the endocannabinoid system in the modulation of food intake and adipogenesis,[11–13] through peripheral mechanisms. The system might provide a possible treatment target for high-risk overweight or obese patients. Insights into the endocannabinoid system have been derived from studies in animals with genetic deletion of CB_1, which have a lean phenotype and are resistant to diet-induced obesity and associated insulin resistance produced by a highly palatable high-fat diet.[14] Further evidence comes from investigation of pharmacological blockade of CB_1 receptors with the selective CB_1 blocker rimonabant, which produces weight loss and ameliorates metabolic abnormalities in obese animals.[10,15] Preclinical findings support the role of the CB_1 receptor in both central and peripheral regulation of energy balance and body weight,[5] providing a mechanistic basis for the clinical development of rimonabant for the management of obesity and associated cardiovascular risk factors.

We undertook a large, multicentre, multi-national, randomised, placebo-controlled trial—the RIO (Rimonabant In Obesity) Europe trial—to assess the efficacy and safety of rimonabant in reducing body weight and improving cardiovascular risk factors in overweight or obese patients.

Methods

Patients

Men and women aged 18 years or older, with body-mass index (BMI) 30 kg/m² or greater, or BMI greater than 27 kg/m² with treated or untreated hypertension or

treated or untreated dyslipidaemia, were recruited from 60 sites in Europe and the USA between October, 2001, and April, 2002. Although RIO-Europe was planned to be done in Europe only, difficulties in meeting recruitment targets led to the extension of the study to 20 sites in the USA with an enrolment, of 276 US patients.

Eligible patients had less than 5 kg variation in bodyweight within the 3 months before study entry. Exclusion criteria included clinical disorders, such as substantial endocrine disease, diabetes mellitus, cardiovascular or pulmonary disease, hepatic and renal disorders, or substantial neurological or psychological illness. Patients were also excluded if they had a history of depression necessitating hospitalisation, two or more recurrent episodes of depression, or suicide attempt. Previous history of surgical procedures for weight loss (eg, stomach stapling, bypass) was also an exclusion criterion. Concomitant use of medications known to alter bodyweight or appetite, including anti-obesity drugs, corticosteroids, antidepressants, neuroleptics, non-selective systemic antihistamines, nicotine substitutes, and antidiabetic drugs, was not permitted. No change in hypolipidaemic medication was allowed. To avoid metabolic effects due to altered smoking habits, patients who indicated their intention to stop smoking were not included. Marijuana and hashish users were excluded from the study.

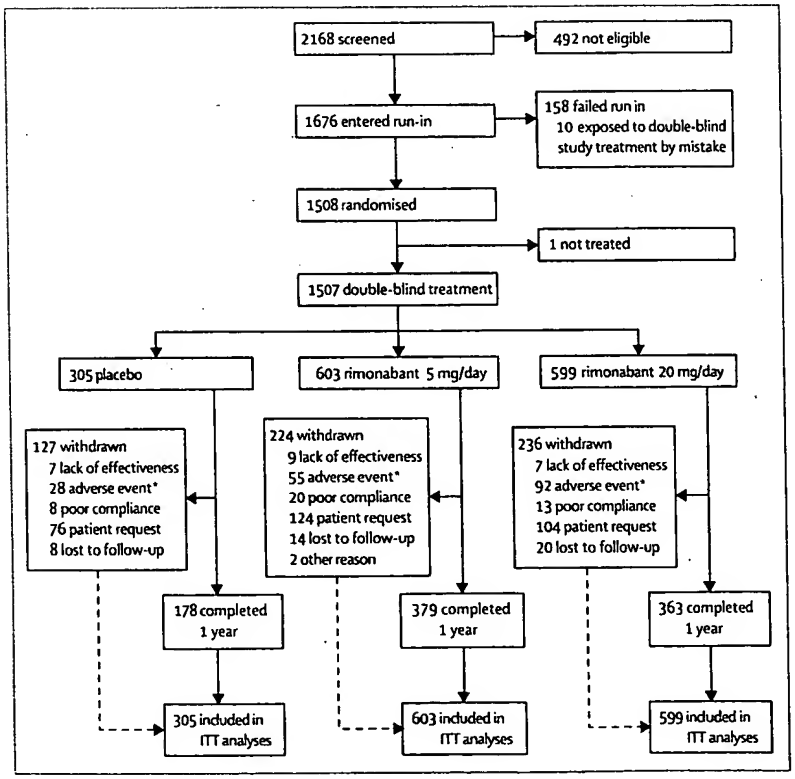

Figure 1: Trial profile
*Including run-in period.

	Placebo (n=305)	Rimonabant 5 mg (n=603)	Rimonabant 20 mg (n=599)
Race (white)*	290 (95·1%)	565 (93·7%)	555 (92·7%)
Sex (female)*	244 (80·0%)	476 (78·9%)	478 (79·8%)
Age (years)†	45·0 (11·6)	45·4 (11·2)	44·6 (11·9)
BMI (kg/m²)†	35·7 (5·9)	36·0 (5·9)	36·2 (5·8)
Weight (kg) †	100·0 (20·3)	100·9 (19·8)	101·7 (19·5)
Waist (cm)†	107·7 (13·8)	108·4 (14·3)	108·8 (14·1)
Hypertension (%)*	116 (38·0%)	264 (43·8%)	237 (39·6%)
Dyslipidaemia (%)*	189 (62·0%)	371 (61·5%)	355 (59·4%)
Metabolic syndrome (%)*	121 (40·6%)	243 (40·8%)	251 (42·4%)
Current smokers (%)*	60 (19·7%)	136 (22·6%)	102 (17·0%)

*Data are number (%). †Data are mean (SD).

Table 1: Baseline characteristics

Procedures

The study was approved by the local ethics committees and done in accordance with the Declaration of Helsinki and ICH Good Clinical Practice between October, 2001, and June, 2004. RIO-Europe was a 2-year randomised, double-blind, placebo-controlled, parallel group, fixed-dose, multicentre study, with a 2-week screening period and 4-week single-blind, placebo run-in period. For the double-blind treatment period, the randomisation code list, with a block size of five, was generated centrally by the sponsor. Treatments were allocated to patients using the interactive voice responding system according to the predefined randomisation list (1: 2: 2 ratio for placebo, 5 mg rimonabant, and 20 mg rimonabant, respectively). A central laboratory (ICON Laboratories, Farmingdale, USA, and Dublin, Ireland) ensured that the randomisation of treatment was balanced within each centre and was stratified based on the loss of bodyweight (≤2 kg or >2 kg) recorded during the run-in period, per protocol. During the double-blind period, patients were seen every 14 days during the first month and thereafter every 28 days until the end of the study.

Basal metabolic rate was estimated with the Harris Benedict formula, and 600 kcal were subtracted by a dietician to calculate a recommended daily energy intake for each patient. At each visit, patients received dietary counselling and were encouraged to increase physical activity.

Bodyweight, waist circumference, and blood pressure were measured at screening, at randomisation, and at every treatment visit, whereas lipid profile, fasting glucose, and insulin were measured every 3 months by use of standard procedures in the central laboratory (ICON Laboratories).[16] Hypertension was defined as systolic/diastolic blood pressure of 140/90 mm Hg or greater. Dyslipidaemia was defined as LDL-cholesterol 3·36 mmol/L or greater, HDL-cholesterol less than 1·03 mmol/L, and triglycerides 1·69 mmol/L or greater. The prevalence of the metabolic syndrome was assessed at screening, baseline, and 12 months, according to the criteria of the National Cholesterol Education Program

Adult Treatment Panel III.[17] An oral glucose tolerance test (75 g glucose) was done at baseline and at 1 year.

The primary efficacy endpoint was the absolute weight change from baseline (randomisation) at the end of year 1 in the intention-to-treat (ITT) population. Another weight-related criterion was the proportion of patients who achieved weight loss of 5% or more and 10% or more. Secondary efficacy endpoints were waist circumference (as a marker of change in abdominal obesity), concentrations of glucose and insulin in serum when fasting, HDL-cholesterol and triglycerides, and the prevalence of the metabolic syndrome. Additional efficacy endpoints were changes in concentrations of total cholesterol and LDL-cholesterol in serum and changes in insulin resistance, derived from the HOMA-IR (homoeostasis model assessment), calculated as fasting insulin (μU/mL)×fasting glucose (mmol/L)/22·5.[18] Analysis of quality of life and dietary assessment were also done at baseline and after 1 year (data still under analysis).

Safety assessments, including physical examination, standard laboratory tests (haematology, liver enzymes, blood chemistry tests), and an ECG, were done at screening, at baseline, and at regular visits every 3 months. Adverse events were recorded at each visit. Mood was evaluated with the Hospital Anxiety and Depression (HAD) scale[19] at baseline and every 3 months. Patients who presented with a symptom of depression or an HAD score of 11 or greater had to be referred to a psychiatrist to ascertain the exact diagnosis of the clinical picture, and treatment if indicated. The HAD score is a short, self-report scale, which is easy to use in a primary-care setting to screen for the presence of mood disorders in different populations of patients, including obese patients.[19] An independent Data Safety Monitoring Board was in place to ensure the safety of the patients by review and analysis of the unblinded safety data, on a regular basis.

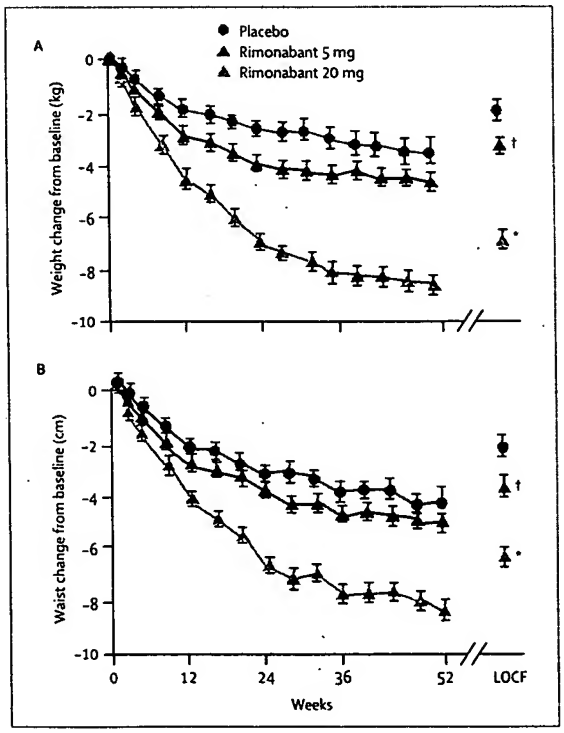

Figure 2: Change from baseline in bodyweight (A) and waist circumference (B)
Data are mean (SE) values for patients completing each scheduled visit, and LOCF (values for the full ITT population with the last observations carried forward).
*p<0·001 *vs* placebo. †p=0·002 *vs* placebo.

	Placebo	Rimonabant		p *vs* placebo	
		5 mg	20 mg	5 mg	20 mg
Weight (kg)					
Baseline	99·9 (20·2)	100·7 (19·7)	101·7 (19·4)		
1 year	98·1 (20·9)	97·3 (20·1)	95·1 (20·6)		
Change	-1·8 (6·4)	-3·4 (5·7)	-6·6 (7·2)	0·002	<0·001
Waist (cm)					
Baseline	107·7 (13·8)	108·3 (14·3)	108·7 (14·1)		
1 year	105·3 (14·3)	104·4 (14·5)	102·2 (15·4)		
Change	-2·4 (6·9)	-3·9 (6·3)	-6·5 (7·4)	0·002	<0·001
SBP (mm Hg)					
Baseline	126·8 (13·7)	127·0 (14·8)	127·0 (14·1)		
1 year	127·0 (13·6)	126·1 (14·7)	126·0 (14·1)		
Change	0·3 (12·3)	-0·9 (12·5)	-1·0 (12·5)	ns	ns
DBP (mm Hg)					
Baseline	79·7 (8·5)	79·6 (9·1)	79·4 (8·8)		
1 year	79·8 (8·7)	78·8 (8·9)	78·5 (8·6)		
Change	0·1 (8·5)	-0·8 (8·8)	-0·9 (8·7)	ns	ns
TC (mmol/L)					
Baseline	5·29 (1·00)	5·37 (0·92)	5·37 (1·00)		
1 year	5·37 (1·01)	5·43 (0·86)	5·42 (0·98)		
Change	0·08 (0·78)	0·06 (0·70)	0·05 (0·70)	ns	ns
HDL-C (mmol/L)					
Baseline	1·27 (0·34)	1·27 (0·32)	1·27 (0·33)		
1 year	1·42 (0·38)	1·46 (0·37)	1·54 (0·40)		
Change	0·15 (0·23)	0·19 (0·23)	0·26 (0·26)	0·048	<0·001
TG (mmol/L)					
Baseline	1·45 (0·87)	1·46 (0·89)	1·45 (0·85)		
1 year	1·43 (0·78)	1·44 (0·92)	1·25 (0·72)		
Change	-0·01 (0·68)	-0·02 (0·77)	-0·20 (0·64)	ns	<0·001
LDL-C (mmol/L)					
Baseline	3·13 (0·82)	3·19 (0·76)	3·21 (0·81)		
1 year	3·30 (0·88)	3·32 (0·75)	3·29 (0·83)		
Change	0·17 (0·70)	0·13 (0·62)	0·08 (0·63)	ns	ns
Total/HDL-C ratio					
Baseline	4·42 (1·28)	4·46 (1·22)	4·44 (1·21)		
1 year	3·99 (1·15)	3·94 (1·11)	3·72 (1·06)		
Change	-0·42 (0·83)	-0·52 (0·80)	-0·71 (0·78)	ns	<0·001
Fasting glucose (mmol/L)					
Baseline	5·26 (0·70)	5·30 (0·62)	5·28 (0·70)		
1 year	5·29 (0·83)	5·26 (0·73)	5·20 (0·68)		
Change	0·03 (0·77)	-0·05 (0·68)	-0·09 (0·65)	ns	0·026
Fasting insulin (mU/mL)					
Baseline	12·4 (9·6)	12·7 (9·2)	12·7 (9·5)		
1 year	14·2 (13·1)	13·0 (10·5)	11·7 (8·3)		
Change	1·8 (13·0)	0·3 (11·2)	-1·0 (8·8)	ns	<0·001
HOMA-IR (%)					
Baseline	3·0 (2·6)	3·1 (2·8)	3·1 (2·5)		
1 year	3·4 (3·5)	3·1 (2·9)	2·8 (2·3)		
Change	0·4 (3·5)	0·0 (3·4)	-0·3 (2·4)	ns	0·002

Data are mean (SD). Analyses for total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), and triglycerides (TG) were done on percentage changes from baseline, and those for cholesterol ratios were done on changes from baseline. SBP=systolic blood pressure. DBP=diastolic blood pressure. ns=not significant.

Table 2: Changes in metabolic and cardiovascular risk factors in ITT population

	Placebo	Rimonabant		p vs placebo	
		5 mg	20 mg	5 mg	20 mg
Weight (kg)					
Baseline	98·5 (19·7)	100·1 (19·6)	102·0 (19·7)		
1 year	94·9 (20·0)	95·4 (19·8)	93·4 (20·8)		
Change	−3·6 (7·4)	−4·8 (6·2)	−8·6 (7·3)	0·042	<0·001
Waist (cm)					
Baseline	108·0 (13·8)	109·0 (14·2)	109·3 (14·4)		
1 year	103·5 (14·3)	103·7 (14·7)	100·8 (15·5)		
Change	−1·5 (7·3)	−5·3 (6·4)	−8·5 (7·4)	ns	<0·001
SBP (mmHg)					
Baseline	127·1 (13·8)	127·4 (14·7)	127·8 (14·1)		
1 year	126·7 (13·7)	126·1 (15·1)	125·8 (13·5)		
Change	−0·4 (12·7)	−1·3 (12·2)	−2·0 (12·6)	ns	ns
DBP (mmHg)					
Baseline	80·2 (8·0)	79·6 (9·3)	79·7 (9·0)		
1 year	79·8 (8·2)	78·2 (9·0)	78·0 (8·5)		
Change	−0·4 (8·1)	−1·5 (8·8)	−1·8 (8·7)	ns	ns
LDL-C (mmol/L)					
Baseline	3·12 (0·81)	3·22 (0·77)	3·18 (0·79)		
1 year	3·33 (0·87)	3·36 (0·75)	3·28 (0·82)		
Change	0·21 (0·70)	0·13 (0·61)	0·10 (0·63	ns	0·024
HDL-C (mmol/L)					
Baseline	1·28 (0·37)	1·26 (0·31)	1·27 (0·33)		
1 year	1·48 (0·41)	1·48 (0·38)	1·59 (0·41)		
Change	0·20 (0·23)	0·23 (0·23)	0·32 (0·26)	ns	<0·001
TG (mmol/L)					
Baseline	1·41 (0·84)	1·45 (0·88)	1·44 (0·80)		
1 year	1·37 (0·69)	1·42 (0·92)	1·18 (0·60)		
Change	−0·04 (0·68)	−0·03 (0·80)	−0·26 (0·60)	ns	<0·001
Fasting glucose (mmol/L)					
Baseline	5·29 (0·76)	5·37 (0·64)	5·31 (0·71)		
1 year	5·30 (0·93)	5·30 (0·68)	5·20 (0·68)		
Change	0·01 (0·90)	−0·07 (0·62)	−0·11 (0·66)	ns	ns
Fasting insulin (mU/mL)					
Baseline	11·8 (7·7)	12·7 (10·3)	12·7 (10·0)		
1 year	12·7 (9·5)	12·5 (8·2)	11·0 (6·1)		
Change	1·0 (8·7)	−0·3 (10·2)	−1·7 (8·8)	ns	0·002
HOMA-IR (%)					
Baseline	2·8 (2·0)	3·1 (3·2)	3·1 (2·7)		
1 year	3·1 (2·5)	3·0 (2·3)	2·6 (1·7)		
Change	0·3 (2·2)	−0·1 (3·3)	−0·5 (2·4)	ns	0·005

Data are mean (SD). Analyses for total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), and triglycerides (TG) were done on percentage changes from baseline. SBP=systolic blood pressure. DBP=diastolic blood pressure. ns=not significant.

Table 3: **Changes in selected metabolic and cardiovascular risk factors in patients who completed 1 year follow-up**

Statistical analysis

For the primary endpoint, analysis was done in the ITT population using the last observation carried forward method and presented as mean and SD, unless otherwise stated. An analysis of variance (ANOVA) model, with treatment and randomisation strata as fixed effects, was used, followed by the modified Bonferroni procedure (Hochberg) to account for multiplicity of doses. For secondary endpoints, continuous variables were analysed by means of one-way ANOVA with treatment as fixed effect. Categorical variables were analysed with the χ^2 test. Each rimonabant dose group was compared with the placebo group.

Analysis of covariance (ANCOVA) and/or logistic regression models using weight loss as covariate were applied to investigate whether the observed effects on efficacy endpoints were independent of weight loss as reflected by the last weight measurement. All statistical tests were two-sided at the 5% significance level.

Role of the funding source

The study was designed by the steering committee, composed of the investigators of the RIO programme and a representative from the sponsor. The trial design and follow-up were assessed by the Trial Operational Committee. Data were collected by the pharmaceutical sponsor and were assessed jointly by the authors and the sponsor. The data were interpreted and the manuscript written by the authors. The corresponding author had full access to all data and had final responsibility for the decision to submit for publication.

Results

309 men and 1198 women were randomised to double-blind treatment. 920 patients (61%) completed the 1-year follow-up: 178 (58·4%) in the placebo group, 379 (62·7%) in the rimonabant 5 mg group, and 363 (60·6%) in the rimonabant 20 mg group (figure 1).

The treatment groups had similar demographic and baseline characteristics (table 1). 346 patients with a BMI of 40 kg/m² or greater were enrolled. At baseline, 617 (40·9%) patients had hypertension, 915 (60·8%) had dyslipidaemia, and 615 (41·4%) met the criteria for metabolic syndrome. During the 4-week run-in period, the mean decrease in weight across all groups was 1·9 kg (SD 2·2), with associated reductions of 1·5 cm (3·5) in waist circumference, 0·05 mmol/L (0·66) in triglyceride concentration, and 0·08 mmol/L (0·23) in HDL-cholesterol concentration.

In the ITT population, change in bodyweight from baseline was significantly greater in the rimonabant 5 mg and 20 mg groups than in the placebo group (figure 2A and table 2). Table 3 shows differences between the groups in patients who completed the allocated treatment. Taking into consideration the mean weight loss during the run-in period of 1·9 kg, total cumulative weight loss ranged from 5 kg in the placebo group to more than 10 kg in patients on rimonabant 20 mg. Waist circumference changed significantly from baseline in the rimonabant 5 mg and 20 mg groups (figure 2B, tables 2 and 3).

Placebo-subtracted analysis showed that rimonabant 20 mg was associated with significant (all $p<0·001$) weight loss (mean −4·7 kg [SE 0·4] for ITT and −5·1 kg [0·6] for completers) and reduction in waist circumference (−4·2 cm [0·5] and −4·0 cm [0·6]; data not shown). In the ITT population, a significantly greater proportion of patients in the rimonabant groups achieved weight loss of 5% or greater from baseline compared with the placebo group (figure 3A). The proportion of completers who had 10% or more weight loss was also greater in the rimonabant 20 mg group than in the placebo group, but not different between the

5 mg group and placebo. A similar pattern of results was seen in completers (figure 3B).

In morbidly obese patients (BMI ≥40 kg/m²), a similar effect on weight loss was recorded compared with the whole study population (data not shown). Results showed no interaction between sex and weight loss: no significant difference in changes was detected between men and women.

Changes in metabolic and cardiovascular risk factors in the ITT population are shown in table 2. In this population, treatment with rimonabant 5 mg and 20 mg increased HDL-cholesterol by 16·2% (SE 0·8; p=0·048 compared with placebo) and 22·3% (0·9; p<0·001), respectively, compared with 13·4% (1·1) in the placebo group (figure 4A). Triglyceride concentrations were reduced by 6·8% (SE 1·5; p<0·0001 *vs* placebo) in the rimonabant 20 mg group, compared with an increase of 5·7% (1·9) in the 5 mg group and 8·3% (2·6) in the placebo group, in the ITT population (figure 4B). Results in completers are presented in table 3.

Logistic regression models and/or ANCOVA using weight loss as a covariate were applied to assess whether the effects of rimonabant 20 mg on both HDL-cholesterol and triglyceride at 12 months were partly independent of weight loss as reflected by the last weight measurement. The weight-loss-adjusted difference in HDL-cholesterol (expressed as the percentage change from baseline) between the placebo and rimonabant 20 mg groups was 3·6% (p=0·01 *vs* placebo), compared with an unadjusted difference of 8·9% (p<0·001 *vs* placebo); this value would translate to about 60% of the increase in HDL-cholesterol being accounted for by the observed weight loss in the ITT population. Similarly, the weight-loss-adjusted difference in the percentage change in triglyceride concentrations between placebo and rimonabant 20 mg was –8·3% (p=0·006 *vs* placebo) compared with the unadjusted difference of –15·1% (p<0·001 *vs* placebo) in the ITT population, corresponding to about 45% of the reduction being accounted for by the observed weight loss.

A significant decrease in non-HDL-cholesterol was observed in the rimonabant 20 mg group compared with placebo (4·3% [SD 16·1] *vs* –0·2% [18·3]; p<0·001) in the ITT population; no difference was noted between the rimonabant 5 mg and placebo groups. Changes in LDL-cholesterol and total cholesterol were not significantly different between the rimonabant and placebo groups.

In the ITT population, 1-year treatment with rimonabant 20 mg resulted in a significant reduction in fasting plasma glucose, compared with the placebo group (table 2). A similar pattern was observed for insulin concentration. A decrease from baseline in HOMA-IR was seen in the rimonabant 20 mg, whereas this index increased in the placebo group. No significant differences in fasting plasma glucose, fasting insulin, or HOMA-IR, were noted between the rimonabant 5 mg group and placebo. Results for completers are presented in table 3. The proportion of patients with impaired

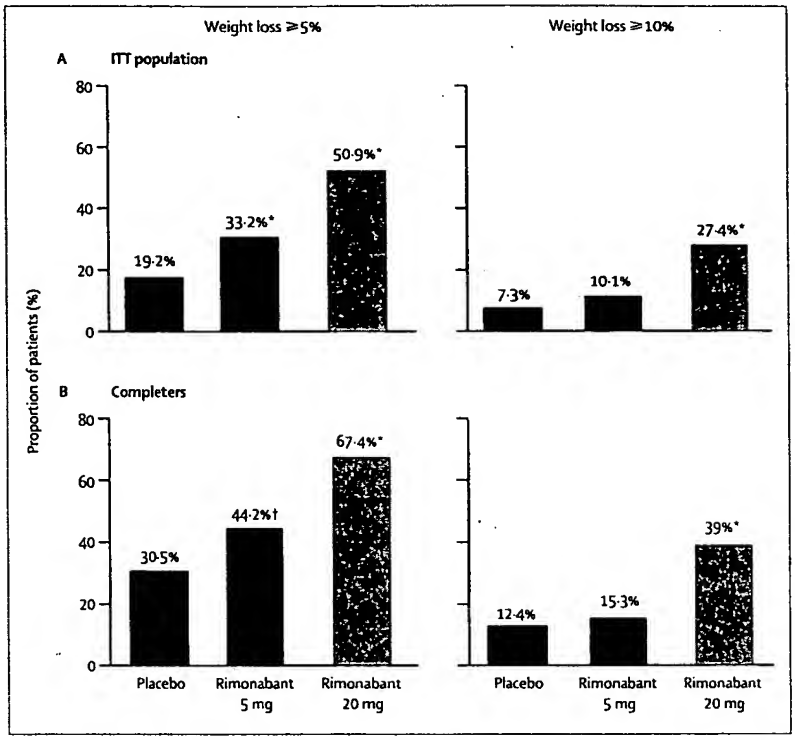

Figure 3: **Proportion of patients who lost ≥5% and ≥10% of baseline weight at 1 year**
*p<0·001 *vs* placebo. †p=0·002 *vs* placebo.

glucose tolerance or diabetes during the oral glucose tolerance test at baseline who improved their glucose tolerance status was not different between groups. The 2-h post-load glucose concentrations were not statistically significant between groups. However, rimonabant 20 mg was associated with a significant reduction in 2-h insulin (–11·0 μU/mL [SD 40·1] from baseline *vs* –2·3 μU/mL [38·5] with placebo; p=0·019), a marker of insulin resistance. There were no significant differences in post-load insulin concentrations between rimonabant 5 mg and placebo.

Overall, there were no interactions between sex and observed weight loss, changes in metabolic parameters, or reduction in waist circumference. Although the systolic and diastolic blood pressure were slightly reduced after 1 year of rimonabant 20 mg treatment, the changes were not significantly different from placebo.

The proportion of patients who fulfilled the criteria for the metabolic syndrome in the ITT and completer populations is shown in table 4. At 1 year from baseline, the proportion had decreased significantly more in the rimonabant 20 mg group than in the placebo group.

The frequency of adverse events was slightly higher in the rimonabant 20 mg group than in the rimonabant 5 mg and placebo groups. Table 5 provides an analysis of all the adverse events occurring in at least 5% of patients in any group. The most common adverse events occurring with rimonabant were: nausea, dizziness,

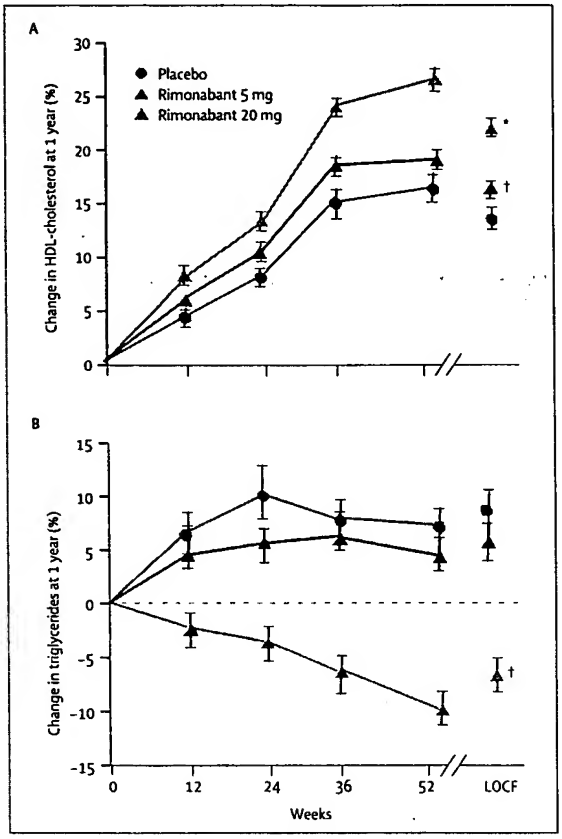

Figure 4: Mean percentage change from baseline in HDL-cholesterol (A) and triglycerides (B)
Data are mean (SE) values for patients completing each scheduled visit, and LOCF (values for the full ITT population with the last observations carried forward). *p<0·001 vs placebo. †p=0·002 vs placebo.

arthralgia and diarrhoea, some patients exhibiting a higher incidence with rimonabant 20 mg. These events, however, were for the most part, mild to moderate in intensity and considered to be transient, based on the occurrence mainly during the first months of the study. There was more headache, fatigue, and upper respiratory infection in the placebo group.

Similar frequencies of serious adverse events were reported in all groups: except for psychiatric disorders, no differences between the treatment groups were observed (tables 5 and 6). Two deaths were reported: one in the placebo group (haemorrhagic cerebrovascular accident, about 2·5 months after randomisation, in a 63-year-old woman treated with phenprocoumon for an aortic valve prothesis), and one in the rimonabant 20 mg group (diagnosis of uterine adenocarcinoma 2 months after randomisation in a 55-year-old woman, resulting in death 3 weeks later due to complications).

The discontinuation rate was similar between the groups, with more withdrawals due to adverse events in the rimonabant 20 mg group and a higher rate of discontinuation due to lack of effect in the placebo group (figure 1). The most common adverse events leading to study discontinuation were depressed mood disorders in all treatment groups; discontinuations due to nausea, vomiting, diarrhoea, headache, dizziness, and anxiety were more frequent in the rimonabant 20 mg group than in the other groups (table 7).

After 1 year, there were no significant changes in the HAD scale subscores for depression (placebo 2·7 [SD 2·9], rimonabant 5 mg 2·7 [2·7], and rimonabant 20 mg 3·4 [3·4]) or anxiety (4·4 [4·0], 4·5 [3·7], and 5·6 [4·1]). Similar proportions of patients with post-baseline depression subscores of 11 or greater were noted in the placebo (23, 8·5%), rimonabant 5 mg (40, 7·5%), and rimonabant 20 mg groups (41, 7·9%). No specific changes in laboratory parameters for haematology or kidney and liver functions were reported. No effect of rimonabant on blood pressure was noted (tables 2 and 3). Mean heart rate remained unchanged from baseline with rimonabant 20 mg, and QTcF decreased by 5·7 msec (SD 16·3) in the placebo group and 3·6 msec (16·9) in the rimonabant 20 mg group.

	Placebo (%)	Rimonabant 5 mg (%)	Rimonabant 20 mg (%)
ITT			
Baseline	108 of 271 (39·9%)	228 of 553 (41·2%)	228 of 540 (42·2%)
1 year	85 of 271 (31·4%)	158 of 553 (28·6%)	106 of 540 (19·6%)*
Change from baseline (%)	21·3%	30·6%	53·6%*
Completers			
Baseline	65 of 167 (38·9%)	155 of 366 (42·3%)	159 of 354 (44·9%)
1 year	43 of 167 (25·7%)	101 of 366 (27·6%)	56 of 354 (15·8%)*
Change from baseline (%)	33·9%	34·8%	64·8%*

*p<0·001 rimonabant 20 mg vs placebo.

Table 4: Prevalence of the metabolic syndrome in the ITT and completer populations at baseline and after 1 year of treatment

Discussion

In this study, treatment with rimonabant over 1 year led to sustained, clinically meaningful weight loss, reduction in waist circumference, and associated improvements in several cardiovascular and metabolic risk factors, including HDL-cholesterol and triglyceride concentrations, HOMA-IR, and prevalence of the metabolic syndrome. About half of the effect of rimonabant on HDL-cholesterol and triglycerides was independent of weight loss. Despite a significant effect on bodyweight, rimonabant 5 mg had an effect of limited clinical interest on metabolic variables. More than 67% of patients who completed treatment with rimonabant 20 mg achieved 5% or more weight loss, and 39% achieved 10% or more weight loss; the target of 5–10% weight loss, which is judged to be standard in the field of conventional obesity treatment, could be achieved.[20,21] The pattern of weight loss observed in this study with rimonabant appears to be sustained up to 36–40 weeks. How this finding would translate into prolonged weight loss in clinical practice has to be determined. The decrease in waist circumference, a measure of abdominal obesity, is known to be

associated with improvements in cardiovascular disease risk factors,[22,23] including atherothrombotic and pro-inflammatory metabolic abnormalities.[24] The weight loss observed in 39% of patients treated with rimonabant 20 mg was associated with a concomitant reduction in waist circumference by about 9 cm, a value that could be associated with a 30% decrease in intra-abdominal adiposity.[24]

Rimonabant treatment was associated with significant improvements in lipid and glycaemic variables. Importantly, the improvements in HDL-cholesterol and triglycerides observed in this study could not be fully explained by the observed weight loss alone; this statement is supported by the changes over time in these metabolic variables compared with bodyweight. The marked increase of HDL-cholesterol among placebo-treated patients can partly be explained by the fact that, during the run-in period, HDL-cholesterol decreased by about 6% (data not shown) as a logical consequence of the negative energy balance during that period. Irrespective of this effect, the placebo-subtracted benefit in HDL-cholesterol increase with rimonabant reached about 10%. In view of the knowledge that a 1% increase in HDL-cholesterol might lead to a 2% reduction in cardiovascular risk, these findings seem to be promising.[25]

The endocannabinoid system is a neuromodulatory system that plays a role in many physiological processes, including the regulation of food intake and energy homoeostasis.[5] Over the past decade, understanding of endocannabinoid biology has progressed substantially with the identification of two G protein-coupled cannabinoid receptors, CB_1 and CB_2,[26,27] and their endogenous ligands. CB_1 receptors are located in the central nervous system and in various peripheral tissues.[28] CB_2 receptors are located in the immune system and do not seem to have a role in energy homoeostasis.[29] Rimonabant is a selective CB_1 blocker that suppresses tonic endogenous activation of the endocannabinoid system centrally[4,6] and peripherally[8,30] (figure 5).

Rimonabant reduces the excessive consumption of palatable food or drinks in rats and marmosets.[31,32] The mechanism by which rimonabant regulates food intake is probably centrally mediated,[15] but recent results suggest an additional peripheral action. Indeed, endocannabinoids derived from the gastrointestinal tract appear to be able to modulate feeding behaviour by acting on CB_1 receptors located on capsaicin-sensitive sensory terminals.[11] In diet-induced obese mice, rimonabant treatment leads to a marked and sustained reduction of bodyweight and adiposity that could not be explained by the transient reduction of food intake observed. When compared with food restriction in a pair-feeding protocol, rimonabant treatment induced a greater bodyweight loss in diet-induced obese mice,[10] indicating that the effects of rimonabant on bodyweight are partly independent of food intake. It seems likely that CB_1 receptors expressed on adipocytes might be one of

	Placebo (n=305)	Rimonabant 5 mg (n=603)	Rimonabant 20 mg (n=599)
Any adverse events	257 (84·3%)	498 (82·6%)	522 (87·1%)
Nasopharharyngitis	48 (15·7%)	87 (14·4%)	93 (15·5%)
Influenza	32 (10·5%)	51 (8·5%)	54 (9·0%)
Gastroenteritis	24 (7·9%)	40 (6·6%)	51 (8·5%)
Upper respiratory tract infection	23 (7·5%)	43 (7·1%)	33 (5·5%)
Bronchitis	16 (5·2%)	34 (5·6%)	34 (5·7%)
Sinusitis	17 (5·6%)	27 (4·5%)	26 (4·3%)
Headache	41 (13·4%)	58 (9·6%)	59 (9·8%)
Dizziness	15 (4·9%)	42 (7·0%)	52 (8·7%)
Nausea	13 (4·3%)	31 (5·1%)	77 (12·9%)
Diarrhoea	9 (3·0%)	36 (6·0%)	43 (7·2%)
Arthralgia	21 (6·9%)	58 (9·6%)	47 (7·8%)
Back pain	26 (8·5%)	56 (9·3%)	55 (9·2%)
Fatigue	17 (5·6%)	24 (4·0%)	25 (4·2%)

Table 5: Patients reporting adverse events (≥5% in any treatment group)

	Placebo (n=305)	Rimonabant 5 mg (n=603)	Rimonabant 20 mg (n=599)
Any serious adverse event	23 (7·5%)	45 (7·5%)	52 (8·7%)
Respiratory disorders	0	0	2 (0·3%)
Psychiatric disorders	1 (0·3%)	2 (0·3%)	9 (1·5%)
Nervous system disorders	3 (1·0%)	7 (1·2%)	3 (0·5%)
Ear disorders	0	0	1 (0·2%)
Cardiac disorders	0	2 (0·3%)	2 (0·3%)
Vascular disorders	0	2 (0·3%)	3 (0·5%)
Gastrointestinal disorders	3 (1·0%)	3 (0·5%)	2 (0·3%)
Hepatobiliary disorders	3 (1·0%)	5 (0·8%)	1 (0·2%)
Musculoskeletal and connective disorders	6 (2·0%)	13 (2·2%)	10 (1·7%)
Renal and urinary disorders	0	2 (0·3%)	2 (0·3%)
Reproductive system and breast disorders	1 (0·3%)	2 (0·3%)	3 (0·5%)
Investigations	1 (0·3%)	0	1 (0·2%)
Injury, poisoning, and procedure complications	4 (1·3%)	5 (0·8%)	4 (0·7%)
Neoplasms: benign, malignant, and unspecified	2 (0·7%)	5 (0·8%)	7 (1·2%)
General disorders	0	0	1 (0·2%)

Data are proportions of patients with at least one serious event.

Table 6: Serious adverse events by system organ class during the double-blind period of the trial

	Placebo (n=305)	Rimonabant 5 mg (n=603)	Rimonabant 20 mg (n=599)
Any adverse event leading to discontinuation	28 (9·2%)	50 (8·3%)	87 (14·5%)
Psychiatric disorders	16 (5·2%)	18 (3·0%)	42 (7·0%)
Depressed mood disorders	9 (3·0%)	14 (2·3%)	22 (3·7%)
Anxiety	1 (0·3%)	0	6 (1·0%)
Agitation	2 (0·7%)	0	3 (0·5%)
Sleep disorders	0	2 (0·3%)	1 (0·2%)
Nervous system disorders	2 (0·7%)	8 (1·3%)	10 (1·7%)
Headache	0	2 (0·3%)	4 (0·7%)
Dizziness	0	2 (0·3%)	2 (0·3%)
Hypoaesthesia	0	0	2 (0·3%)
Gastrointestinal disorders	0	5 (0·8%)	21 (3·5%)
Nausea	0	1 (0·2%)	14 (2·3%)
Vomiting	0	0	4 (0·7%)
Diarrhoea	0	0	3 (0·5%)
Dyspepsia	0	0	2 (0·3%)
Flatulence	0	2 (0·3%)	0
Cardiac disorders	3 (1·0%)	2 (0·3%)	5 (0·8%)
Palpitations	1 (0·3%)	0	2 (0·3%)

According to the Medical Dictionary for Regulatory Activities in at least two patients in any treatment group (one patient may report several events). Only main system organ classes are presented.

Table 7: Patients reporting adverse events leading to discontinuation

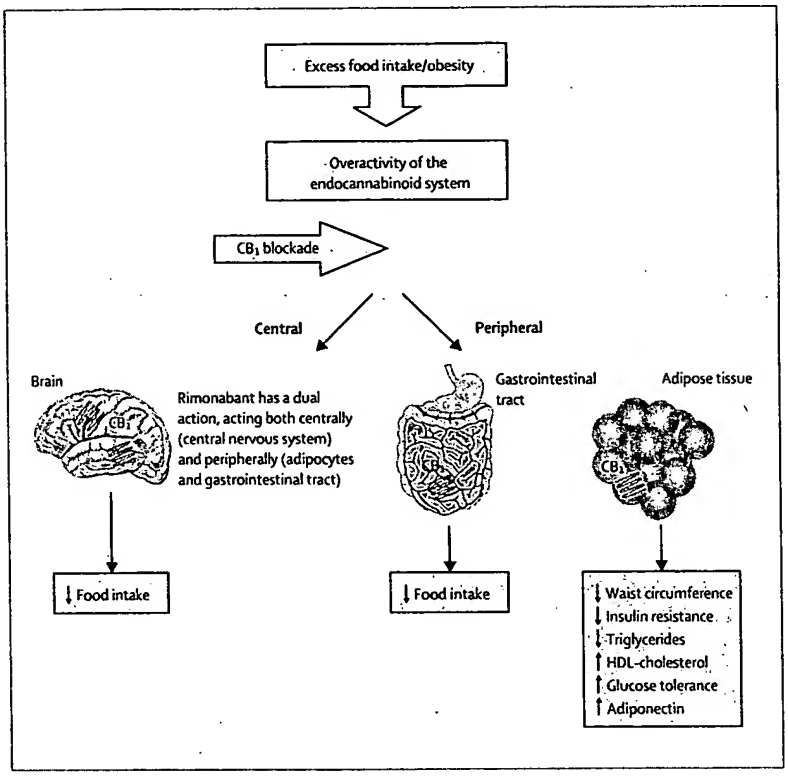

Figure 5: **Hypothetical model of role of central and peripheral components of endocannabinoid system in regulation of food intake and peripheral metabolism**
CB_1 receptors are enriched in regions of the brain and in gastrointestinal system implicated in the regulation of food intake, and in adipose tissue. CB_1 receptor blockade might contribute to decreased food intake and exert direct metabolic effects.

the effectors of the possible peripheral metabolic action of rimonabant.

A possible explanation for the potential weight-independent effect of rimonabant on HDL-cholesterol and triglycerides might be related to the observation that rimonabant enhances the mRNA expression of adiponectin, an adipokine secreted by fat cells and reported to have a role in the regulation of hyperglycaemia, hyperinsulinaemia, and fatty acid oxidation,[11-16] at the peripheral adipocyte level.[8] Thus, improved fat-cell function may be postulated as a key peripheral effect of rimonabant leading to bodyweight reduction and improvement in metabolic parameters, including lipids and beneficial changes in adiponectin and C-reactive protein. Further studies of the in-vivo effects of the increased adiponectin production induced by rimonabant treatment are needed to elucidate possible metabolic effects of rimonabant in adipose tissue.

Rimonabant treatment was well tolerated during this trial, with a similar overall drop-out rate in all treatment groups. The most common adverse events experienced with rimonabant 20 mg, such as nausea and diarrhoea, were found to be mild and generally occurred in the first few months of the treatment. Gastrointestinal side-effects might be explained by the mechanism of action of the drug, since it is known that CB_1 receptors are present in the gut and likely to be involved in gastrointestinal motility. Serious adverse events did not seem to occur more frequently in the patients treated with rimonabant than in those on placebo. Mood disorders were more frequent in the rimonabant 20 mg treatment group than in the other groups, but the discontinuation rate due to this adverse event was similar between rimonabant 20 mg and placebo in this study.

The RIO-Europe trial was designed to reflect a real-life clinical setting in which we assessed parameters indicative of the metabolic syndrome and relevant clinical endpoints, such as waist circumference, in patients with a range of pre-existing risk factors. The 1-year results emphasise that blockade of the CB_1 receptor clearly targets several causes of cardiovascular risk, including obesity and the metabolic syndrome, along with its associated parameters such as waist circumference, HDL-cholesterol, and insulin resistance. The prevalence of the metabolic syndrome, compared with baseline, was reduced by more than half in the ITT population and by almost two-thirds in completers. There has been an increased awareness of the importance of this syndrome and its relation to cardiovascular disease in recent years. The large number of patients treated with CB_1 blockade who achieved the 10% target for weight loss or had a marked improvement in the top risk factors established by the world-wide INTERHEART study,[17] suggests that rimonabant can be considered as a valuable adjunct therapy for weight and waist reduction in patients at high cardiovascular risk.

The finding of a significant reduction in the incidence of the metabolic syndrome after 1 year of treatment with rimonabant 20 mg could have further implications, since the metabolic syndrome has been shown to be an important predictor of the development of type 2 diabetes and coronary heart disease.[38,39] However, the long-term benefits of weight loss and treatment of the metabolic syndrome on the prevention of cardiovascular events and mortality have yet to be confirmed by long-term outcomes studies.

In conclusion, the results of the RIO-Europe trial indicate that modulating the activity of the endocannabinoid system by blocking its CB_1 receptors holds therapeutic promise as an approach to the treatment of obesity and associated risk factors. Treatment with rimonabant was associated with clinically meaningful weight loss and additional improvements in waist circumference, lipid concentrations, and insulin resistance, and had a favourable safety profile.

Contributors
L Van Gaal and A J Scheen were involved in the study design and study follow-up as members of the RIO Operational Committee. Data and final analysis were reviewed and validated by all authors, who then wrote the manuscript. L Van Gaal had full unrestricted access to the complete set of data and wrote the initial draft of the paper. All the named authors participated in the study and contributed to interpretation of data and

revision of the manuscript. The final version was written by L Van Gaal and A J Scheen, and was seen and approved by all authors.

RIO-Europe Study Group

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References

1 James PT, Rigby N, Leach R. The obesity epidemic, metabolic syndrome and future prevention strategies. *Eur J Cardiovasc Prev Rehabil* 2004; 11: 3–8.

2 York DA, Rossner S, Caterson I, et al. American Heart Association. Prevention conference VII: obesity, a worldwide epidemic related to heart disease and stroke: Group I: worldwide demographics of obesity. *Circulation* 2004; 110: e463–70.

3 WHO. Integrated management of cardiovascular risk: report of a WHO meeting. Geneva; World Health Organisation, 2002.

4 Cota D, Marsicano G, Tschop M, et al. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest* 2003; 112: 423–31.

5 Di Marzo V, Bifulco M, De Petrocellis L. The endocannabinoid system and its therapeutic exploitation. *Nat Rev Drug Discov* 2004; 3: 771–84.

6 Di Marzo V, Goparaju SK, Wang L, et al. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 2001; 410: 822–25.

7 Croci T, Manara L, Aureggi G, et al. In vitro functional evidence of neuronal cannabinoid CB1 receptors in human ileum. *Br J Pharmacol* 1998; 125: 1393–95.

8 Bensaid M, Gary-Bobo M, Esclangon A, et al. The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Mol Pharmacol* 2003; 63: 908–14.

9 Howlett AC, Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Porrino LJ. Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology* 2004; 47 (suppl 1): 345–58.

10 Ravinet Trillou C, Arnone M, Delgorge C, et al. Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am J Physiol Regul Integr Comp Physiol* 2003; 284: R345–53.

11 Gomez R, Navarro M, Ferrer B, et al. A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. *J Neurosci* 2002; 22: 9612–17.

12 Kirkham TC. Endogenous cannabinoids: a new target in the treatment of obesity. *Am J Physiol Regul Integr Comp Physiol* 2003; 284: R343–44.

13 Horvath TL. Endocannabinoids and the regulation of body fat: the smoke is clearing. *J Clin Invest* 2003; 112: 323–26.

14 Kunos G, Batkai S. Novel physiologic functions of endocannabinoids as revealed through the use of mutant mice. *Neurochem Res* 2001; 26: 1015–21.

15 Cota D, Marsicano G, Lutz B, et al. Endogenous cannabinoid system as a modulator of food intake. *Int J Obes Relat Metab Disord* 2003; 27: 289–301.

16 Laboratory Test Handbook. 4th edn. Hudson: Lexi-Comp, 1996.

17 Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation* 2002; 106: 3143–421.

18 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412–19.

19 Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* 1983; 67: 361–70.

20 Goldstein DJ. Beneficial health effects of modest weight loss. *Int J Obes Relat Metab Disord* 1992; 16: 397–415.

21 Van Gaal LF, Wauters MA, De Leeuw IH. The beneficial effects of modest weight loss on cardiovascular risk factors. *Int J Obes Relat Metab Disord* 1997; 21 (suppl 1): S5–9.

22 Zhu S, Wang Z, Heshka S, Heo M, Faith MS, Heymsfield SB. Waist circumference and obesity-associated risk factors among whites in the third National Health and Nutrition Examination Survey: clinical action thresholds. *Am J Clin Nutr* 2002; 76: 743–49.

23 Janssen I, Katzmarzyk P, Ross R. Waist circumference and not body mass index explains obesity-related health risk. *Am J Clin Nutr* 2004; 79: 379–84.

24 Després JP, Lemieux I, Prud'homme D. Treatment of obesity: need to focus on high risk abdominally obese patients. *BMJ* 2001; 322: 716–20.

25 Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation* 1989; 79: 8–15.

26 Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 1990; 346: 561–64.

27 Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 1993; 365: 61–65.

28 Howlett AC. The cannabinoid receptors. *Prostaglandins Other Lipid Mediat* 2002; 68–69: 619–31.

29 Petrocellis LD, Cascio MG, Marzo VD. The endocannabinoid system: a general view and latest additions. *Br J Pharmacol* 2004; 141: 765–74.

30 Liu Y, Connoley I, Wilson C, Stock M. Effects of the cannabinoid CB1 receptor antagonist SR141716 on oxygen consumption and soleus muscle glucose uptake in Lepob/Lepob mice. *Int J Obes Relat Metab Disord* 2004; 29: 183–87.

31 Arnone M, Maruani J, Chaperon F, et al. Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology (Berl)* 1997; 132: 104–06.

32 Simiand J, Keane M, Keane PE, Soubrie P. SR 141716, a CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset. *Behav Pharmacol* 1998; 9: 179–81.

33 Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 2001; 7: 947–53.

34 Combs TP, Berg AH, Obici S, Scherer PE, Rossetti L. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J Clin Invest* 2001; 108: 1875–81.

35 Fruebis J, Tsao TS, Javorschi S, et al. Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci USA* 2001; 98: 2005–10.

36 Yamauchi T, Kamon J, Waki H, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; 7: 941–46.

37 Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 2004; 364: 937–52.

38 Meigs JB, Wilson PW, Nathan DM, D'Agostino RB Sr, Williams K, Haffner SM. Prevalence and characteristics of the metabolic syndrome in the San Antonio Heart and Framingham Offspring Studies. *Diabetes* 2003; 52: 2160–67.

39 Alexander CM, Landsman PB, Teutsch SM, Haffner SM. NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes* 2003; 52: 1210–14.

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Endogenous cannabinoids and appetite

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Since pre-history, *Cannabis sativa* has been exploited for its potent and manifold pharmacological actions. Amongst the most renowned of these actions is a tendency to provoke ravenous eating. The characterization of the psychoactive principals in cannabis (exogenous cannabinoids) and, more recently, the discovery of specific brain cannabinoid receptors and their endogenous ligands (endocannabinoids) has stimulated research into the physiological roles of endocannabinoid systems. In this review, we critically discuss evidence from the literature that describe studies on animals and human subjects to support endocannabinoid involvement in the control of appetite. We describe the hyperphagic actions of the exogenous cannabinoid, Δ^9-tetrahydrocannabinol, and the endogenous CB1 ligands, anandamide and 2-arachidonylglycerol, and present evidence to support a specific role of endocannabinoid systems in appetitive processes related to the incentive and reward properties of food. A case is made for more comprehensive and systematic analyses of cannabinoid actions on eating, in the anticipation of improved therapies for disorders of appetite and body weight, and a better understanding of the biopsychological processes underlying hunger.

Endocannabinoids: Anandamide: Reward: Appetite regulation

Introduction

The past few decades have witnessed a dramatic growth in our knowledge of important central and peripheral factors affecting the regulation of appetite. This is particularly the case with respect to the neurochemical components of brain systems influencing ingestive behaviours. Each advance in pharmacological analysis has brought with it a bevy of new candidate neurotransmitters which have been found to have significant influences on what, or how much, we eat (e.g. Cooper & Clifton, 1996; Hoebel, 1997; Holst, 1999; Schwartz *et al.* 2000). Nevertheless, the growth in the number of agents known to affect feeding behaviour has not

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Abbreviations: GABA, γ-aminobutyric acid; THC, tetrahydrocannabinol.

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been fully matched by a better understanding of the psychological processes subserved by the interactions of these substances with their receptors. At a physiological level, the abundance of putative chemical 'signals' suggests either that there is considerable redundancy, or the existence of interactive systems so complex that real understanding of the role of each component is likely to take many more decades to unravel.

When considering the pharmacology of appetite, it is the case that most research effort has been diverted into the search for biochemical signals that terminate feeding. Such 'satiety signals' have been emphasized, in large part, because of the increased prevalence of obesity and the profits to be made from treatments that specifically target the physiological processes that reduce appetite. Although several neurotransmitter systems have been identified which may play a role in the stimulation of eating (e.g. Stratford *et al.* 1998; Edwards *et al.* 1999), appetite research is now largely devoted to the analysis of agents which reduce food intake. The study of the neurochemical processes that give rise to appetite has thus been overshadowed, to the detriment of progress in the broader understanding of appetite regulation and, more generally, the biological bases of fundamental motivational mechanisms.

Considering appetite for food, it seems logical to consider that man has evolved to maximize every opportunity to eat, in anticipation that times of feast will be followed by periods of famine. We have evolved a psychological predisposition to overconsume; responding to food variety, being over-sensitive to the high palatability of energy-dense foods, and possessing a seemingly limitless capacity to store excess energy in adipose tissue. Moreover, psychological factors such as conditioning and expectation seem to be far more influential in determining our eating patterns than predominant homeostatic models of energy and body-weight balance might argue (Mela & Rogers, 1998). In this context, a plethora of mechanisms designed to limit our intake, or to maintain our body weights at some arbitrary 'set point' imply a level of redundancy that is contrary to biological necessity. Similarly, arguments that widespread obesity might arise from some genetic defect seem, on the whole, unfounded. We only have to consider the average BMI of individuals in the USA compared with their cousins in the old world to see that geography and opportunity have generally been far more influential than genes.

In modern societies, it is our susceptibility to the sight, taste, or thought of food which impels overconsumption and (when matched with easy availability of food and reduced energy expenditure) gives rise to overweight and obesity. Therefore, the overshadowing of appetitive processes by the overwhelming study of satiety mechanisms is clearly deserving of some redress. A greater knowledge of the neurochemical factors underlying the urge to eat, or the pleasure derived from eating will have crucial implications for understanding general motivational processes, as well as having far-reaching clinical implications. To this end, we now turn our discussion to one of the most recently discovered neurochemical families: the endogenous cannabinoids. In the following text, we will present data to implicate these substances in the normal biopsychological mechanisms which create appetite and stimulate eating.

Cannabis sativa, exogenous cannabinoids and endocannabinoids

Cannabis sativa (Indian Hemp, marijuana) has been cultivated for at least 10 000 years, in part to obtain fibres for the manufacture of textiles and rope, but to an important extent because of the wide variety of pharmacological actions that follow ingestion or inhalation of the leaves or resin extracts. These pharmacological actions include ataxia, hypothermia, analgesia, short-term memory deficits, a sense of time dilation, enhanced sensation, euphoria and higher-order cognitive impairments (Dewey, 1986; Hollister, 1986). These various effects gave rise to the

historical medicinal use of cannabis and underlie its contemporary recreational abuse. Recent research has added to these, principally psychological actions of cannabis, evidence for a number of beneficial effects, including: suppression of cancer cell proliferation, analgesia, alleviation of glaucoma, antioxidant actions, stabilization of the symptoms of multiple sclerosis (e.g. Nahas *et al.* 1999; Di Marzo *et al.* 2000; Hampson *et al.* 2000; Pertwee, 2000). Even the seeds of *Cannabis sativa* have been promoted as a useful source of *n*-3 and *n*-6 fatty acids.

The chemicals within cannabis that produce biological effects in people were not characterised until 1964, when Gaoni and Mechoulam isolated Δ^9-tetrahydrocannabinol (THC) and a group of related organic 'cannabinoid' molecules in hashish (Gaoni & Mechoulam, 1964; Mechoulam *et al.* 1970). With subsequent developments in neuropharmacology, scientists were able to demonstrate that these cannabinoids exerted their effects via specific sites within the central nervous system and peripheral tissues. We now know that there is a family of G-protein linked, cell-surface cannabinoid receptors (Devane *et al.* 1988; Matsuda *et al.* 1990; Munro *et al.* 1993). Two main cannabinoid receptors subtypes have been identified and their genes cloned (Onaivi *et al.* 1996). These are classified as a 'central-type' CB1, widely distributed within the central nervous system and many peripheral tissues, and a 'peripheral-type' CB2 receptor, which is not significantly expressed in the central nervous system (Breivogel & Childers, 1998). It is generally agreed that the behavioural effects of cannabinoids are mediated by brain (CB1) cannabinoid receptors and, despite their wide distribution, the regional localization of receptors corresponds closely with their behavioural effects (Herkenham *et al.* 1990; Breivogel *et al.* 1997).

The existence of specific receptor sites mediating the effects of plant-derived exogenous cannabinoids suggested the existence of a chemical produced within mammalian tissues, for which the cannabinoid receptors are the target; endogenous ligands which compounds like THC mimic to produce their various effects. After several decades of unsuccessful searching, the 1990s saw the isolation and complete characterisation of the first 'endocannabinoid' (Devane *et al.* 1992). This compound, arachidonylethanolamide, which is synthesised within brain tissue and binds with high affinity to CB1 receptors, was named anandamide, from 'ananda', a Sanskrit word meaning inner bliss. Subsequently, the search for additional endogenous ligands selective for the CB2 cannabinoid receptor led to the identification of 2-arachidonoylglycerol (Mechoulam *et al.* 1995; Stella *et al.* 1997). Although it exhibits a lower affinity for CB1 receptors than anandamide, evidence suggests that it is present in the brain at higher levels than anandamide and is a full agonist at CB1 receptors (Stella *et al.* 1997). Both of these substances fulfil the necessary criteria for classification as neuromodulators: they are synthesised from arachidonic acid through distinct biosynthetic routes; are released from neurons in response to membrane depolarization, have specific uptake mechanisms and are hydrolysed by a selective enzyme, fatty acid amide hydrolase (Di Marzo & Deutsch, 1998; Piomelli *et al.* 1998; Reggio, 1999). Cannabinoids are also closely related to the arachidonic acid-derived eicosanoids, and may have overlapping physiological functions (Burstein *et al.* 1995; Fimiani *et al.* 1999). A number of other candidate endocannabinoids have since been characterised, but anandamide and 2-arachidonoylglycerol are considered to be the primary ligands at CB1 and CB2 receptors, with both substances capable of exerting THC-like effects in animal behavioural models (for reviews, see Pertwee, 1995; DiMarzo & Deutsch, 1998; Di Marzo *et al.* 1998). Importantly, amphibian, rodent and human CB1 receptors show a high degree of homology. Together with the occurrence of the endocannabinoids in a number of phylogenetically diverse species, a high degree of evolutionary conservation of cannabinoid signalling systems indicates that they should play an important physiological role in vertebrate brain function (De Petrocellis *et al.* 1999; Soderstrom *et al.* 2000).

Cannabinoids and appetite

The discovery of the endocannabinoids leads to the obvious question: what is their normal physiological function? As noted earlier, administration of cannabinoids induces a wide spectrum of behavioural and physiological changes. It is likely that these effects, produced by stimulation of cannabinoid receptors, reflect the normal roles of endocannabinoid systems.

Marijuana use in man has long been associated with an increase in appetite, with references to appetite stimulant properties recorded as early as AD 300 (Abel, 1975). Modern cannabis users are also very familiar with the drug's capacity to provoke eating and overconsumption, a predictable phenomenon known colloquially as 'the munchies'. However, despite the verity of hyperphagia as represented in pharmacopoeia over many centuries and in modern anecdote, there is a real paucity of detailed scientific analysis of the phenomenon. The initial discovery of the exogenous cannabinoids led to some fairly cursory examination of their therapeutic applications in the alleviation of appetite loss associated with disease states (Abel, 1971; Greenberg *et al.* 1976; Foltin *et al.* 1986, 1988). However, there has still been no comprehensive, systematic characterisation of cannabinoid effects on feeding in human subjects. Similarly, the animal literature data is very insubstantial compared with that for other drugs or neurotransmitters that affect eating. Disappointingly, there have been few consistent findings and very little research in the past 20 years. Clinical development of cannabinoid treatments, and the development of theoretical models of their actions on appetite, calls for a concerted research effort to replace our dependence on anecdotal accounts.

Contemporary progress in cannabinoid pharmacology means that we now have the proper tools to examine endocannabinoid involvement in appetite regulation, and the first real opportunity to develop effective cannabinoid therapies for disorders of appetite or body weight. In the following text, we will give an overview of the current state of knowledge concerning cannabinoids and feeding and present our own theoretical account of the role of endocannabinoids in appetite.

Exogenous cannabinoids and human appetite

As we pointed out earlier, hyperphagia following cannabis intoxication is a widely accepted phenomenon, but one which was for many years supported only by anecdotal reports (Haines & Green, 1970; Tart, 1970; Halikas *et al.* 1971). The first scientific studies we have found took place in the1970s, but since then only a handful of reports have entered the literature.

In 1971, Hollister examined the effects of oral Δ^9-THC (0·5 mg/kg) in fasted subjects, offered chocolate milk shakes at 30 min intervals over 2 h (Hollister, 1971). The drug significantly increased intake at each time point, with no evidence of the gradual fall in consumption seen after placebo. These changes were accompanied by consistently higher hunger ratings and greater appreciation of food as judged by appetite questionnaires. Similar effects on intake were obtained in a second experiment with unfasted subjects, although changes in hunger and appetite scores were more variable. Abel (1971) reported that inhalation of two cannabis cigarettes (of unknown potency) caused subjects to eat as many as fifty marshmallows, compared with only four by control subjects (the actual time over which the eating took place is not reported). As we shall see later, apparently selective effects of cannabinoids on palatable, and particularly sweet, ingesta have been very influential in later theoretical accounts of the role of endocannabinoids in feeding. But it should be noted that in the Abel (1971) experiment, there was no choice of foods, and the selection of a sweet confection as a test food was entirely serendipitous.

In the first systematic study of cannabinoid effects on feeding, Greenberg *et al.* (1976) examined long-term body-weight changes, dietary selection and energy intake in marijuana smokers tested under research ward conditions over the course of 1 month. In contrast to previous studies, dosage was well-defined, with subjects receiving marijuana cigarettes containing approximately 20 % Δ^9-THC. However, subjects were allowed to smoke freely, with the number of marijuana cigarettes smoked per d gradually increasing over the course of the experiment. In subjects who were experienced marijuana users, daily energy intake rose from a pre-drug level of 3200 (SE 200) kcal (13·39 (SE 0·84) MJ), to peak in the first few days of treatment at 3900 (SE 300) kcal (16·32 (SE 1·26) MJ), before declining to an average of 3300 (SE 200) kcal/d (13·81 (SE 0·84) MJ/d). Overeating was matched by a persistent body-weight increase, averaging 2·3 kg across the entire 21 d drug phase. In fact, body weight continued to rise despite the stabilization of energy intake. During a 5 d post-drug phase, both body weight and energy intake decreased dramatically. Subjects lost an average of 1.8 kg as daily energy intake fell by as much as 1000 kcal (4·18 MJ). Unfortunately, no data was provided on the specific changes in eating patterns or food selection associated with these changes.

The next real advance came from studies by Foltin and colleagues in the 1980s (Foltin *et al.* 1986, 1988). In their 1986 study, subjects were tested in a relatively naturalistic, residential laboratory for periods of up to 25 d. Each test day comprised three phases: a private work period, a performance task and a period of social access. Active or placebo marijuana cigarettes (containing 1·84 or 0 % Δ^9-THC respectively) were smoked before private work periods and during social access. Average daily intake increased from 2780 (SE 130) kcal (11·63 (SE 0·54) MJ) under placebo conditions to 3340 (SE 160) kcal (13·97 (SE 0·67) MJ) with the active marijuana treatment. Interestingly, the overconsumption primarily occurred during periods of social access, with subjects consuming an average of 2500 kcal (10·46 MJ) compared with 1000 kcal (4·18 MJ) under placebo conditions. Notably, this excess was obtained by an increase in the frequency and consumption of 'snack foods', rather than of the set meals provided each day.

In a subsequent experiment Foltin specifically tested the effects of smoked marijuana (1·3 or 2.3 % Δ^9-THC) on the intake of different foods (Foltin *et al.* 1988). Subjects were provided with a wide variety of different food items which they could eat at will. Marijuana increased total food intake by doubling the number of snacks. The main increase in energy intake was largely attributed to an increase in the intake of sweet solid snack items such as candy bars, cookies and cakes. The intake of sweet drinks (e.g. cola, fruit juice), or savoury solid items (e.g. potato chips) were less susceptible. Overall, the distribution of energy from carbohydrate, fat and protein did not differ between drug and placebo conditions.

Similar effects of Δ^9-THC on food selection were also reported by Mattes *et al.* (1994), who compared the relative hyperphagic potency of Δ^9-THC administered via acute oral administration, smoke inhalation or suppository. Relatively small increases in energy intake were derived principally from increased snack consumption, rather than self-selected meals.

Clinical applications of exogenous cannabinoids

While cannabinoid-induced appetite stimulation (or, more strictly, increased food intake) has been demonstrated under laboratory conditions, the few studies performed leave many questions unanswered. More controlled studies are clearly required to determine just how the motivation to eat is affected by cannabinoids, and to establish whether overconsumption is a general phenomenon or is specifically tied to particular taste modalities or specific foods. An additional

component of action of Δ^9-THC is that it exerts an antiemetic effect (Gralla, 1999) which may, or may not, be related to the drug's stimulation of appetite. Moreover, this action is not limited to Δ^9-THC. For example, Abrahamov *et al.* (1995) reported that Δ^8-THC, an analogue of Δ^9-THC with fewer psychotropic actions, was found to abolish vomiting in child patients simultaneously treated with anti-cancer drugs. Such findings indicate that broader analysis of the effects of the different cannabinoids are warranted to establish their behavioural specificity, with the possibility of developing therapeutic applications which lack some of the undesired psychological side effects of Δ^9-THC.

Despite the limitations of the human studies discussed earlier, it is clear that Δ^9-THC has potential to induce substantial elevation of food intake and promote body-weight gain, with the possible additional benefit of limiting the nausea and vomiting associated with many chemotherapy regimens. It is therefore unsurprising that clinicians were keen to assess the utility of cannabinoid treatments in relation to clinical syndromes involving appetite or weight loss. A few, somewhat limited, studies have been conducted with Δ^9-THC to examine the drug's capacity to ameliorate low appetite and wasting in clinical populations with cancer cachexia or HIV (Cat & Coleman, 1994; Gorter, 1999). One of the earliest trials, by Regelson *et al.* (1976), found that oral Δ^9-THC doses of up to 15 mg/d stimulated appetite and produced significant weight gain in advanced cancer patients. In a single-case report, Sacks *et al.* (1990) examined the effect of Δ^9-THC on food intake during a highly emetigenic chemotherapy regimen. Treatment with Δ^9-THC alone (5 mg, three times per d) had little effect on intake, but greatly attenuated the severe reduction in daily energy intake produced by chemotherapy (intake + Δ^9-THC 1453 kcal (6·08 MJ); without Δ^9-THC 764 kcal (3·32 MJ)). In contrast to the findings of Foltin and Mattes (Foltin *et al.* 1986, 1988; Mattes *et al.* 1994), this difference was attributed largely to an increase in energy derived from fat. No meaningful changes in appetite ratings were noted.

Plasse *et al.* (1991) reported the effects of chronic Δ^9-THC treatment in patients with HIV-wasting syndrome. The drug was given at total daily doses of 5–20 mg for as long as 20 weeks (administered orally at 2·5 mg twice per d, or 5 mg four times per d). Δ^9-THC not only reduced nausea, but increased both appetite and mood ratings. Of the patients who responded to the treatment, the majority gained weight, while those that continued to lose weight did so at a slower rate than previously. Drug treatment was accompanied by a weight gain of up to 5·8 kg/month (median 0·54 kg/month), compared with a median weight loss of 0·93 kg/month in the 3 months before treatment.

In a randomized double-blind study of five AIDS patients, Struwe *et al.* (1993) tested the effects of 5 mg Δ^9-THC, given twice per d before meals. Small increases in appetite scores, energy intake and body weight were accompanied by significant increases in body fat. In addition, the patients showed a marked improvement in symptom distress scores and mood. These latter effects raise a further issue which must be addressed experimentally: the extent to which changes in appetite in wasting patients are the result of specific cannabinoid actions on appetite, or to more generalized changes in their sense of well-being.

In a more comprehensive, multi-centre survey, Beal *et al.* (1995) evaluated the long-term effects of Δ^9-THC or placebo in eighty-eight patients with AIDS-related appetite and weight loss. Dronabinol (2·5 mg, twice per d before lunch and supper) was administered over 42 d. Patients who had previously suffered progressive weight loss experienced either stabilization of their body weight or a modest weight gain. In patients who were unaffected by concurrent illness, body weight increased by as much as 1·1 kg. Accompanying these changes were substantial increases (38 %) in appetite ratings across the whole course of treatment. Self-ratings of mood and nausea were also improved. Interestingly, improvements in mood preceded the

changes in appetite and nausea ratings, again indicating the need for more thorough assessment of the wider psychological and behavioural effects of these treatments.

On the basis of studies such as these, the commercial preparations of Δ^9-THC (dronabinol; trade name, Marinol) or a synthetic analogue (Cesamet) have been licensed for clinical use in the treatment of chemotherapy-induced nausea and the treatment of AIDS-associated anorexia and weight loss. It is likely that the number of therapeutic applications could be extended as beneficial effects are reported in other clinical areas. For example, it was recently reported that daily dronabinol treatment in dementia patients, whose symptoms included refusal to eat, produced significant weight gain (but not, paradoxically, significant increases in energy intake; Volicer, 1997).

However, we are still faced with a marked absence of substantial data for the efficacy of cannabinoids in reversing disease- or chemotherapy-related changes to appetite or body-weight status. It seems clear to us that medicinal application of these drugs could be optimized through a better understanding of their actions on the psychological and behavioural components of appetite. Especially important is the need for data on the extent to which the different actions of the drugs (antiemetic, appetite stimulation, mood enhancement) account for the beneficial effects; or indeed whether those effects are separable. With recent advances in cannabinoid pharmacology it is now possible to overcome some of the limitations of human studies by a detailed examination of cannabinoid interventions in animal models. As we detail later, new findings using such models are beginning to pinpoint the precise mechanisms underlying cannabinoid-induced hyperphagia.

Cannabinoid effects in animal models

Much like the literature on human subjects, the database on cannabinoids and feeding in animals is rather insubstantial. A brief spate of studies followed the isolation of the exogenous cannabinoids and the availability of Δ^9-THC, but from the late 1970s until the discovery of anandamide this was a neglected area of investigation. More importantly, the advantages of animal (predominantly rat) models in allowing manipulation of dose and test conditions were overlooked, and only the most cursory analyses of the behavioural actions of exogenous cannabinoids on feeding were carried out. Less than optimal experimental designs also resulted in the majority of studies either failing to find any effect on eating, or actually inducing anorectic effects through the use of high, narcotic doses (e.g. Sofia & Barry, 1974; Graceffo & Robinson, 1998). Abel (1975), reviewing the earliest animal experiments with cannabis extracts or Δ^9-THC, found that out of a total of twenty-five experiments published between 1965 and 1975, only three reported increased intake.

One of the first studies to demonstrate hyperphagia in rats was performed by Glick & Milloy (1972). They reported that an intraperitoneal dose of 1·0 mg Δ^9-THC/kg produced a modest increase in food intake (less than 2 g over 2 h). Unfortunately, their study was compromised through the imposition of 24 h food and water deprivation prior to testing. Under such circumstances, which represent an extreme and rather unnatural physiological challenge, it is difficult for animals to express hyperphagia above the voracious eating already induced by starvation.

Using more naturalistic methods, Brown *et al.* (1977) found that lower, orally administered doses of Δ^9-THC (0·25 or 0·4 mg/kg) significantly increased intake of both food and a palatable 0·8 M-sucrose solution. However, these increases were again very modest: food intake was increased by less than 1 g in a 1 h test; sucrose solution consumption was raised by approximately

7 ml. Like Abel's (1971) 'marshmallow effect' in people, this apparently greater effect on sweet ingesta has subsequently been cited as evidence of a preferential action of cannabinoids on palatable food intake. However, we should reiterate the fact that the increases reported by Brown *et al.* (1977) were relatively minor compared with effects that may be obtained in human subjects. Moreover, apparently selective drug effects on particular foods or flavours may reflect existing preferences rather than a particular mode of action. Indeed, it has been shown that apparently selective effects of drugs on macronutrient intake actually derive from an individual animal's preference for particular foods at the time the drug takes effect (Gosnell *et al.* 1990).

Finally, we should note that not all research has been restricted to the laboratory rat. For example, McLaughlin *et al.* (1979) found that intravenous injection of 0·5 and 1·0 mg/kg Δ^9-THC produced hyperphagia in sheep. Foreshadowing the later discovery of cannabinoid receptors, these workers also found that the effect was stereospecific: only the L-, and not the D-isomer, of Δ^9-THC induced eating (McLaughlin *et al.* 1979).

In the absence of thorough behavioural analyses of Δ^9-THC effects on feeding in rats, we undertook a comprehensive series of tests to better characterize the drug's actions. We initially adopted a pre-feed paradigm in which the rats were thoroughly sated by the provision of a palatable wet mash meal before drug administration. This procedure ensures low baseline intakes and so maximizes our ability to detect hyperphagia. We also conducted our tests during the dark period of a daily 12 h light–dark cycle, as rats are predominantly nocturnal feeders. After oral Δ^9-THC administration, the animals were given unrestricted access to their normal maintenance diet (more usually referred to as 'lab chow' (g/kg): protein 163, fat 29, carbohydrate 460). As can be seen in Fig. 1, a wide range of doses stimulated eating (Williams *et al.* 1998). Furthermore, the maximum effect of the drug was far greater than any previously reported. A 1·0 mg/kg dose produced a greater than 4-fold increase in consumption over 1 h. After administration of the highest dose, non-specific behavioural effects of the drug were evident, such as impaired motor coordination and sedation. Above this dose, the acute, motoric and sedative side effects are such that animals are incapable of overeating.

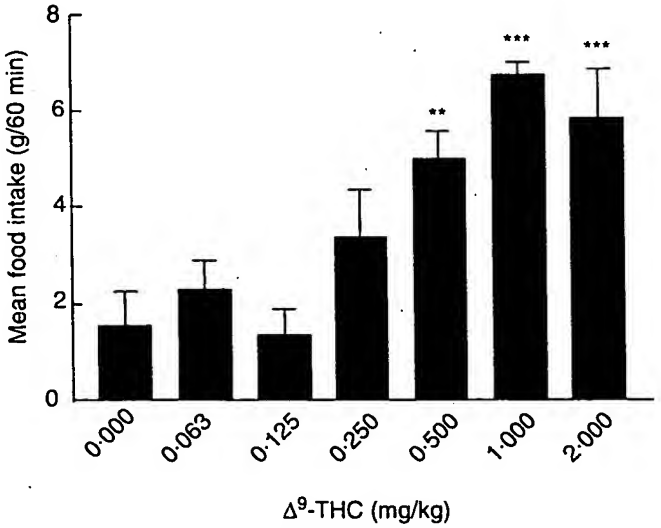

Fig. 1. Orally administered Δ^9-tetrahydrocannabinol (THC) exerts a dose-dependent hyperphagic action in pre-satiated rats (rats consumed approximately 30 g palatable wet mash during 2 h before drug administration). The eating produced by this exogenous cannabinoid can be attenuated by pre-treatment with the selective CB1 receptor antagonist, SR141716 (Williams *et al.* 1998). Values are means with standard errors shown by vertical bars. Mean values were significantly different from vehicle condition: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

Subsequent experiments confirmed that these hyperphagic effects of Δ^9-THC were mediated by central CB1 cannabinoid receptors. Specifically, we found that hyperphagia was significantly attenuated by SR141716, a selective CB1 receptor antagonist, but not by SR144258, a selective antagonist of the peripheral type, CB2 receptor (CM Williams and TC Kirkham, unpublished results).

One aspect of these data that deserves particular emphasis is the magnitude of the overconsumption that was induced by Δ^9-THC. Our pre-fed animals were thoroughly satiated, having already eaten an amount of wet mash equivalent to their normal daily food intake (> 20 g). The substantial intake that followed Δ^9-THC treatment thus signifies that stimulation of CB1 receptors can provoke an exceptionally powerful stimulus to eat. Moreover, the extent of Δ^9-THC-induced overeating was similar, if not in fact greater than, that induced by central administration of the neurotransmitter neuropeptide Y (for example, see Clark *et al.* 1984), widely regarded as being a key component of the brain mechanisms that promote ingestive behaviour. The remarkable potency of Δ^9-THC, and the attenuation of its hyperphagic effects by CB1 blockade, thus provides a very convincing case for involvement of the endocannabinoid systems in the normal regulation of feeding.

An important aspect of modelling cannabinoid effects in animals is the examination of chronic effects of the drug. If we are to obtain an adequate understanding of the behavioural changes that might affect clinical applications of the cannabinoids, long-term studies are essential. Again, the literature on chronic Δ^9-THC administration generally relates to the use of relatively high doses of the drug, and the most consistent report is of anorectic consequences. A typical finding is that food intake is persistently suppressed with a consequent reduction in body weight (Manning *et al.* 1971; Sjoden *et al.* 1973; Sofia & Barry, 1974). These effects are probably due to the narcotic properties of the doses employed (Drewnowski & Grinker, 1978). Chronic cannabinoid treatment thus remains an important, and currently neglected, area of study. In our own pilot experiments, mimicking typical clinical treatment regimens, we have found that while Δ^9-THC will produce acute increases of food intake rats tend to display rebound hypophagia, compensating for their initial overconsumption. Consequently, over 24 h, total intake may be suppressed relative to controls. When measured over several days, the net consequence of daily (single or multiple) Δ^9-THC doses is to actually retard the normal weight gain in rats fed *ad libitum*. Further experiments are thus required to determine the optimum level and frequency of dosing to obtain persistent elevation of intake and body weight.

While the potent CB1-mediated hyperphagic effects of Δ^9-THC provide strong evidence of endocannabinoid involvement in appetite, this possibility necessitates the demonstration that the endogenous cannabinoids, themselves, will also exert hyperphagic actions. The contemporary literature provided no support for this possibility, with the only previous test of anandamide effects on eating being unsuccessful (Crawley *et al.* 1993). We nevertheless conducted our own investigation, using the same pre-feed design as had been so effective for THC. In our tests, anandamide was indeed found to significantly increase chow intake in pre-fed rats (Williams & Kirkham, 1999*a*), over a range of peripherally administered doses (Fig. 2). Although the degree of overeating was quite modest compared with the effects of Δ^9-THC, the most effective dose (1 mg/kg) produced a doubling of intake over a 3 h test. Moreover, anandamide hyperphagia was entirely prevented by pre-treatment with SR141716, while the CB2 antagonist SR144258 was without effect (Fig. 3), indicating that the overeating was specifically mediated by central CB1 receptors.

More intriguingly, anandamide effects were apparent over a much longer time course than those of Δ^9-THC. Whether this reflects continued bioavailability of the exogenously administered endocannabinoid (which is very susceptible to enzymic degradation once within neurons),

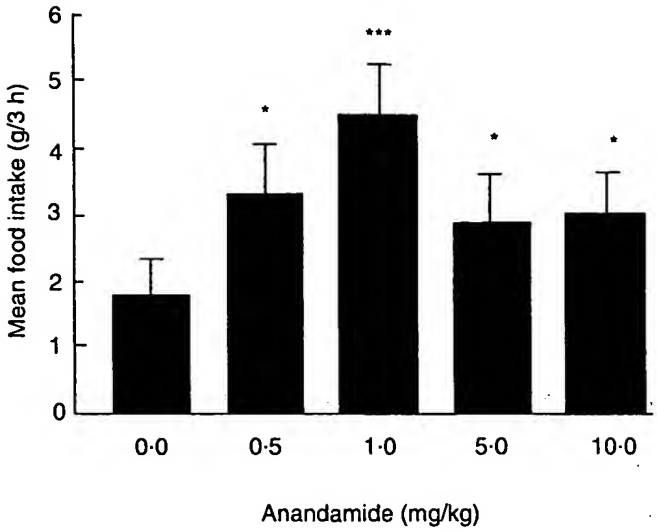

Fig. 2. Subcutaneous injection of the endogenous cannabinoid, anandamide, produces significant overeating in pre-fed rats. The degree of hyperphagia is modest compared with the effects of Δ^9-tetrahydrocannabinol, but has a much longer duration (Williams & Kirkham, 1999). Values are means with standard errors shown by vertical bars. Mean values were significantly different from vehicle condition: $^*P < 0{\cdot}05$, $^{***}P < 0{\cdot}001$.

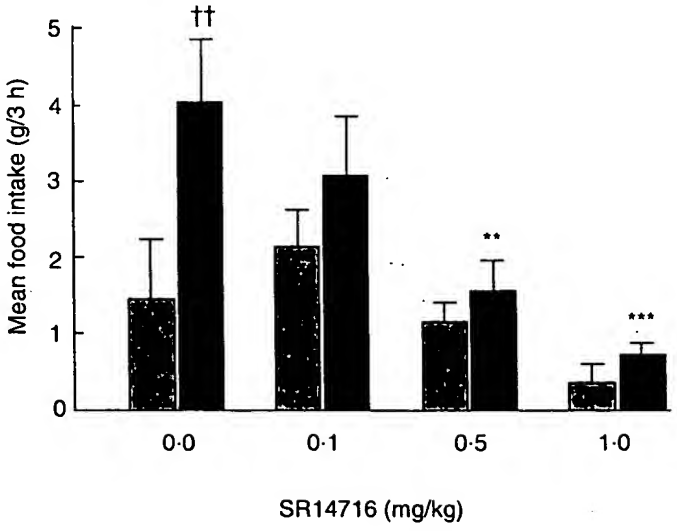

Fig. 3. Hyperphagia in anandamide-treated, pre-fed rats (■) is prevented by pretreatment with the selective CB1 antagonist, SR141716 (▩), indicating that the effects are mediated by central cannabinoid receptors. The grey bars display weak effects of SR141716 when administered alone. Values are means with standard errors shown by vertical bars. Mean value was significantly greater after subcutaneous anandamide (1·0 mg/kg): $\dagger\dagger P < 0{\cdot}01$. Mean values showed significant attenuation of anandamide hyperphagia: $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

or involves the initiation of a cascade of physiological events remains to be determined. However, what was striking was that the largest effects occurred at times when, under control conditions, animals were most likely to engage in substantial feeding. Thus, early in the test when the satiating effect of the pre-feed was apparent as an almost complete suppression of

feeding, anandamide produced only very weak effects. Only when the inhibitory effects of the pre-feed began to wane did the stimulatory actions of the endocannabinoid become easily apparent. We are tempted to speculate that our data represent an amplification, or potentiation, of endocannabinoid activity associated with the normal, episodic pattern of meal-taking in rats.

Importantly, anandamide-induced feeding has also been replicated in mice by Hao *et al.* (2000) with a very low dose of the cannabinoid (0·001 mg/kg, intraperitoneally). In addition, as a corollary to these agonist data, we should also note that repeated, daily administration of the CB1 antagonist, SR141716, has been shown to suppress appetite and induce persistent weight loss in rats (Colombo *et al.* 1998). Although tolerance to the drug's effect on appetite was apparent after 5 d, the resultant suppression of body-weight gain was evident across the full course of a 14 d experiment. These combined agonist and antagonist data thus provide the first clear indications that endogenous cannabinoid systems may play a normal role in the physiological regulation of appetite.

Behavioural characterization of cannabinoid hyperphagia: the reward hypothesis

The preceding findings do provide evidence for some role of endocannabinoids in appetite regulation. However, increases in food intake alone tell us little about what aspect, or aspects, of the motivation to eat are actually altered to affect behavioural change. Indeed, given the wide pharmacological spectrum of cannabinoids, it is essential to demonstrate that hyperphagia follows from a natural adjustment to feeding motivation, and not through some non-specific action (we will return to this issue later). Certainly, the very limited insights provided by the early cannabinoid research demand far more detailed analyses of behavioural modifications induced by CB1 ligands before detailed hypotheses can be formed.

Despite the lack of precise information about cannabinoid actions, one hypothesis has gained particular influence. As we noted earlier, early reports from some studies with animals and human subjects were suggestive of a more marked susceptibility of palatable foods to the stimulant effects of Δ^9-THC. While those data are not wholly convincing (and may be artifactual), they have given rise to the notion that cannabinoids may provoke overconsumption by amplifying the orosensory reward, or palatability, of foods (Arnone *et al.* 1997).

A common feature of drugs of abuse is their activation of the brain pathways which normally subserve the appetitive and consummatory aspects of natural rewards, such as food and sex. There is convincing evidence to suggest that the exogenous cannabinoids can also influence these brain reward systems (for review, see Gardner & Vorel, 1998). Reward circuitry can be also be activated by electrical stimulation of the brain: animals will actually perform complex instrumental tasks to obtain such stimulation. Moreover, with appropriate environmental stimuli, electrical brain stimulation can also induce and sustain those behaviours, such as eating, which normally produce reward. It is significant then that Trojniar & Wise (1991) were able to show that Δ^9-THC will facilitate feeding induced by electrical stimulation of the lateral hypothalamus (a brain region long associated with feeding and reward processes). These effects imply that Δ^9-THC amplifies the rewarding properties of food with a consequent increase in the motivation to eat.

Endocannabinoids are also implicated in food reward by the behavioural effects of CB1 blockade. Arnone and colleagues reported that in rats and marmosets SR141716 selectively attenuated the consumption of palatable ingesta (Arnone *et al.* 1997; Simiand *et al.* 1998), while having little or no effect on bland food intake (of the kind normally provided to laboratory animals for their general maintenance). These workers suggested that such preferential

effects of CB1 blockade indicate important tonic endocannabinoid activity underlying food reward. Thus, cannabinoid agonists could increase food intake by rendering foods more palatable, while antagonists might tend to diminish the hedonic value of foods, and so reduce consumption.

With these data in mind, we began a series of studies to directly address the cannabinoid-reward hypothesis and, more generally, to obtain more thorough details of cannabinoid effects on feeding behaviour. As a starting point, we began by measuring the effects of SR141716 on sucrose sham-feeding. In this model, rats are surgically implanted with a chronic gastric fistula (a sealable cannula through which gastric contents may be drained). The animals ingest palatable sucrose solutions, which are recovered within seconds directly from the stomach. Under these circumstances, normal satiation mechanisms are minimized and ingestion is motivated exclusively by food palatability (Weingarten & Watson, 1982). In the absence of normal satiety, sham-feeding rats will consume many times the amount of sucrose solution ingested by intact, normally-feeding rats. Moreover, the rate of sham-feeding is proportional to the palatability of the sucrose: the sweeter the solution, the more avid the ingestive response. Consequently, the model is particularly sensitive to manipulations that affect orosensory reward.

We hypothesized that, if endogenous cannabinoids directly mediate food reward, sham-feeding should be disrupted by CB1 blockade. More specifically, we anticipated that suppression of sham-feeding by SR141716 would produce changes in behaviour which resemble the effect of diluting the sucrose solution (Kirkham & Cooper, 1988; Kirkham, 1990). A precedent for such an effect comes from our previous work with opioid antagonists. Opioids are heavily implicated in orosensory reward (see later), in part through the demonstration that opioid receptor antagonists reduce sucrose sham-feeding in a manner which exactly mimics the changes in ingestion produced by sucrose dilution, and hence the palatability, of the sucrose. In addition, attenuation of sham-feeding by opioid antagonists can be reversed by increasing the palatability of the sucrose during a sham-feeding test (Kirkham & Cooper, 1988; Kirkham, 1990; Leventhal *et al.* 1995).

Contrary to our expectations, SR141716 failed to affect sucrose sham-feeding at all (Kirkham & Williams, 1998). Even doses ten times greater than those that reverse Δ^9-THC- or anandamide-induced feeding (Williams & Kirkham, 1999*a*; CM Williams, PJ Rogers and TC Kirkham, unpublished results), or reportedly suppress sucrose drinking in intact animals (Arnone *et al.* 1997) were ineffective. The failure of SR141716 to attenuate sham-feeding argues strongly against significant endogenous cannabinoid activity within the pathways which maintain sucrose ingestion. In other words, endocannabinoids do not seem to be primarily involved in food reward during ingestion, and are not crucial to the pleasure derived from orosensory characteristics of food.

However, while our sham-feeding data do not support endocannabinoid mediation of the consummatory aspects of food reward, our data do not entirely preclude their involvement in some other aspect of feeding-related reward processes. It is possible, for example, that endocannabinoids are associated with appetitive, or incentive, aspects of feeding motivation, related to the anticipation of food or the desire to eat.

Going beyond the feeding literature to studies of endocannabinoid involvement in alcohol craving, we can find convincing evidence that cannabinoid interventions do indeed modify appetitive–incentive processes. McGregor and his colleagues have reported several experiments using an operant, lick-based, progressive ratio paradigm as a model of craving. Rats are required to complete a progressively greater number of responses (licks at a spout) to obtain successive reinforcements of small volumes (0·1 ml) of some liquid (typically alcohol or sucrose solutions). The reinforcement ratio at which animals cease to respond (the 'break-

point') is taken as an index of the degree of craving. Gallate & McGregor (1999) found that the CB1 antagonist SR141716 produced a dose-related reduction in break-point to obtain beer reinforcers. By contrast, they found that a CB1 agonist, CP 55940, would increase break-points in rats licking for beer or sucrose solutions; i.e. rats would work harder to obtain reinforcement (Gallate *et al.* 1999). These effects, which were also reversed by SR141716, strongly implicate endocannabinoid systems in the processes underlying the motivation to obtain palatable ingesta.

Returning to feeding, we have also obtained data to support endocannabinoid involvement in incentive motivation. Using an open-field apparatus, we observed the behaviour of satiated rats following administration of Δ^9-THC and anandamide. Under control conditions rats generally displayed little motivation to eat. When eating did occur, it did so only after many minutes engaged in exploratory behaviours (Fig. 4). By contrast, both exogenous and endogenous cannabinoid treatments stimulated feeding, dramatically reducing the latency to eat. Crucially, once initiated, the subsequent pattern of feeding behaviour displayed by Δ^9-THC- and anandamide-treated rats in the open field is identical to that of untreated rats feeding freely in their home cages (Williams & Kirkham, 1999*b*; CM Williams, unpublished results). At this stage of our investigations, we cannot entirely preclude non-specific actions of the cannabinoids which might account for these latency effects (for example, an anxiolytic action which could suppress exploratory activity in favour of eating). However, in no case was there any evidence of unnatural activities (such as stereotypy) that might otherwise account for the increased feeding. With these cautions in mind, we feel that these data are compatible with an action of cannabinoids to increase the incentive value of the food and advance the species-typical sequence of feeding behaviours.

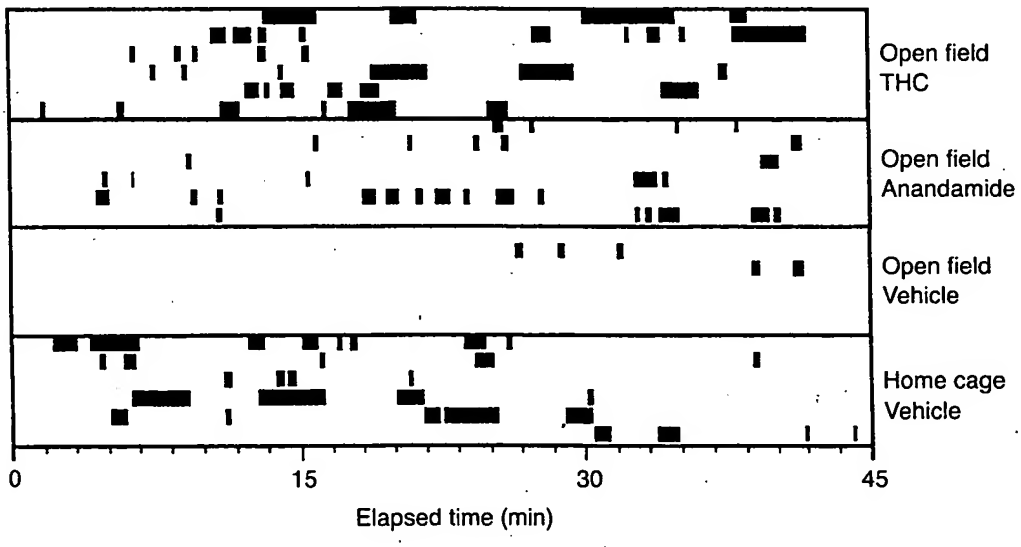

Fig. 4. These charts represent the occurrence of eating episodes in groups of pre-satiated rats, observed either in their home cage after saline injection, or in an open field arena following administration of hyperphagic doses of Δ^9-tetrahydrocannabinol (THC), anandamide or vehicle. For each condition, behavioural traces are presented for six rats, allowing comparison of feeding behaviour after vehicle and each drug dose. In a relatively novel open field, vehicle-treated rats show little motivation to eat. However, both Δ^9-THC and anandamide actively promote eating: reducing the latency to begin eating and inducing a pattern of feeding which is very similar to that seen in the untreated rats under home cage conditions.

We have also observed these effects using a more naturalistic, continuous meal pattern monitoring technique, where moment-to-moment feeding is monitored in the animals' home cages (for review of meal pattern methodology, see Clifton, 2000). Under these circumstances, the latency to the first meal of pre-satiated or free-feeding rats is consistently reduced after endocannabinoid administration; often by more than 1 h compared with the control values.

Interestingly, a similar action of Δ^9-THC on eating latency was reported by Trojniar & Wise (1991) in their experiments on eating induced by electrical stimulation of the lateral hypothalamus. Together with the Gallate *et al.* (1999) break-point data, such findings imply that stimulation of CB1 receptors increases the salience of food and hence the motivation to eat. We thus begin to see the development of a model which links endocannabinoids directly to the processes that lead to the initiation of feeding. Recalling the lack of effect of cannabinoid receptor blockade on the intrameal palatability factors which maintain sham-feeding, these data tend to support a specific endocannabinoid involvement in the inter-meal motivational processes that culminate in meal taking.

Further support for this notion comes from our pilot experiments examining interactions between food deprivation and the anorectic potency of SR141716 on the intake of bland laboratory chow (TC Kirkham and CM Williams, unpublished results). The antagonist was administered to rats which had been food deprived for 18 h or maintained on a schedule of restricted access to food. We found that whereas rats fed *ad libitum* were unaffected by the drug, restricting food availability resulted in significant intake suppression by SR141716. These preliminary results suggest that deprivation induces, or enhances, endocannabinoid activity. As SR141716 acts as a competitive antagonist at CB1 receptors, the behavioural effects of CB1 blockade will only become apparent if there is endogenous cannabinoid release and receptor stimulation. The greater the level of cannabinoid activity, the greater will be the behavioural consequences of SR141716 treatment.

In effect, the stimulatory actions of the cannabinoids on eating resemble the physiological changes which occur with food deprivation, since both manipulations reduce eating latency and promote short-term increases in meal size (Bivens *et al.* 1998). Unfortunately, this aspect of our story is complicated by a report that SR141716 fails to affect operant responding for food pellets in food-restricted rats (Rodriguez de Fonseca *et al.* 1999); precisely the circumstances where antagonist-induced reduction of responding might be expected. There is clearly much more work to be done to resolve these issues, but it is interesting that Hao *et al.* (2000) have recently reported that doses of anandamide that can provoke overeating in mice also reverse some of the changes in brain neurotransmitter turnover that are induced by food restriction.

Nevertheless, on the basis of our data, we might propose that endogenous cannabinoid activity gradually increases during intermeal intervals to reach some critical level when motivation to eat is triggered. Accordingly, the longer the time that has elapsed since the last meal, the greater will be the activity in relevant endocannabinoid circuits, and the higher the motivation to eat. We might also assume that there are natural rhythms in such activity which are correlated with normal patterns of meal taking, so that the optimal demonstration of CB1 agonist or antagonist effects on feeding will be obtained by carefully synchronizing drug administration with these endogenous cycles.

Interactions between cannabinoids and brain reward systems

The notion that endocannabinoids are involved in appetitive aspects of feeding is compatible with the known effects of Δ^9-THC on brain reward pathways. Central to these pathways are the

mesolimbic dopaminergic neurons, arising in the ventral tegmental area and projecting to the nucleus accumbens (Spanagel & Weiss, 1999). Natural rewards, including food, together with many drugs of abuse, have been found to stimulate dopamine release from terminals in the nucleus accumbens. Researchers now emphasize a specific role for these pathways in incentive motivation, i.e. the generation of emotional arousal and behavioural activation in response to stimuli, which predict reward (Ikemoto & Panksepp, 1999; Spanagel & Weiss, 1999; Berridge, 2000).

Ingestion of food causes dopamine release in the nucleus accumbens, especially after deprivation, or if the food is novel or palatable. In addition, food restriction is known to enhance the rewarding properties of food and of drugs of abuse (Berridge, 1991; Cabeza de Vaca & Carr, 1998). It is perhaps not coincidental, then, that doses of Δ^9-THC which we have found to produce hyperphagia have also been found to stimulate dopamine release in the nucleus accumbens (Gardner, 1992; Tanda *et al.* 1997; Gardner & Vorel, 1998; Williams *et al.* 1998).

Other data indicate that the various behavioural effects of CB1 agonists can be modified by dopamine receptor antagonists (Souilhac *et al.* 1995; Sanudo-Pena *et al.* 1996), and that cross tolerance can occur between CB agonists and dopamine agonists (Rodríguez de Fonseca, 1994). In addition, CB1 receptors have been found to be co-localized, and to interact, with dopamine D1 and D2 receptors (Bidaut-Russell & Howlett, 1991; Glass *et al.* 1997). Overall, there is growing support for functional relationships between endocannabinoid and dopaminergic activity in the brain. Therefore, it is entirely feasible that brain dopaminergic systems implicated in general incentive processes and drug craving could also be involved in the feeding effects of cannabinoids. It will be important, therefore, to assess the extent to which feeding effects of CB1 ligands can be affected by treatments modifying dopamine function.

In addition to dopamine, the endogenous opioid peptides are also linked to central reward processes. For example, in the accumbens, dopamine neurons synapse with enkephalinergic neurons that are critical to the expression of reward- or incentive-related behaviours (Gardner & Vorel, 1998). In the ventral tegmental area, opioids are thought to remove mesolimbic dopaminergic neurons from γ-aminobutyric acid (GABA)-mediated inhibition (Spanagel & Weiss, 1999).

Evidence has also accumulated to support overlapping endogenous opioid and endocannabinoid mechanisms in relation to a wide range of physiological processes (Fuentes *et al.* 1999), including reward and appetite. For example, CB1 receptor knockout mice are not only unresponsive to cannabinoids, but display a reduced sensitivity to the rewarding properties of opiate drugs (Ledent *et al.* 1999). In addition, in the same strain of mice, morphine administration fails to stimulate the nucleus accumbens dopamine release found in animals that do express the CB1 receptor, suggesting that CB1 receptors regulate mesolimbic dopamine transmission (Mascia *et al.* 1999).

Gardner *et al.* (1988) reported that enhancement of brain-stimulation reward by Δ^9-THC was blocked by the general opioid receptor antagonist, naloxone. Importantly, the facilitation by Δ^9-THC of feeding induced by stimulation of the lateral hypothalamus is also blocked by naloxone (Trojniar & Wise, 1991). Finally, Gallate *et al.* (1999) found that the facilitatory effects of a CB agonist on responding for palatable solutions were reversed by both a CB1 antagonist and naloxone. Such findings imply that cannabinoids modulate the motivation to ingest via actions on both cannabinoid and opioid systems.

Opioids are firmly implicated in the mediation of food reward in their own right by the ability of opioid receptor agonists and antagonists to increase or reduce food intake respectively. These effects have been shown to involve changes in the hedonic evaluation of foods

(for reviews, see Kirkham & Cooper, 1991; Cooper & Kirkham, 1993). For example, opioid antagonists are reported by human subjects to reduce the perceived palatability of previously preferred foods and fluids. Since food palatability is one of the principal determinants of the persistence of eating, it is important that opioid manipulations primarily affect the duration of meals and are most apparent in tests with palatable foods.

Although our analyses are in their preliminary stages, the meal pattern analyses referred to earlier suggest that there may be a secondary action of anandamide. In addition to the marked effects on eating latency, there may also be a tendency for agonist administration to increase the size and/or duration of meals. If this latter effect can be confirmed, it would suggest that the behavioural consequences of cannabinoid treatments may not, after all, be restricted solely to intermeal incentive processes. Given the potent influence of food palatability on the maintenance of eating, any tendency of cannabinoids to increase meal duration would suggest some enhancement of orosensory reward. However, it is also possible that any effect of cannabinoids on intrameal factors may be indirect, and mediated through interactions with other important neurochemical systems mediating food palatability. Some of our recent experiments do indeed provide convincing evidence for interactions between cannabinoids and endogenous opioids in relation to feeding.

To investigate this possibility, we first attempted to block the hyperphagic actions of Δ^9-THC with naloxone. We found that even low, subanorectic doses of the opioid antagonist effectively blocked cannabinoid-induced overconsumption (CM Williams and TC Kirkham, unpublished results). Subsequently, we examined whether combined administration of the CB1 antagonist SR141716 with naloxone could provide further evidence of co-operative interactions between cannabinoid and opioid systems. We chose a range of doses of each antagonist which, alone, are capable of reversing the actions of agonists at their respective binding sites but exert no significant effect on chow intake. As can be seen in Fig. 5, neither naloxone alone, nor

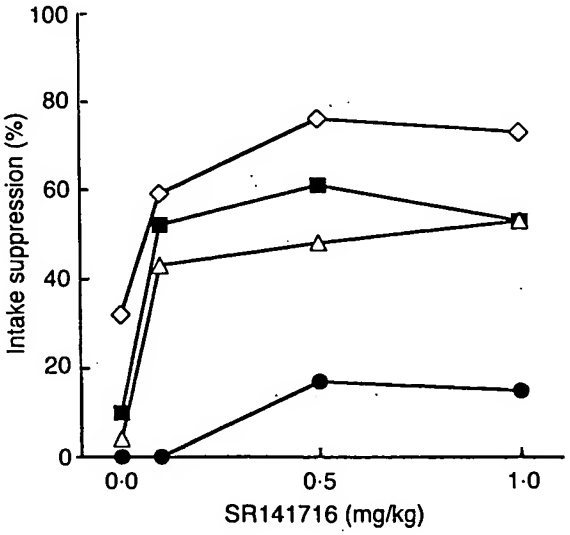

Fig. 5. Subanorectic doses of the opioid antagonist naloxone and the CB1 antagonist, SR141716, were given alone or in combination to non-deprived rats. Each line indicates the effect of a single dose of SR141716 with increasing doses of naloxone (NX). (●), 0·0 mg NX/kg; (△), 0·1 mg NX/kg; (■), 0·5 mg NX/kg; (◇), 1·0 mg NX/kg. The data are presented as intake suppression (%) relative to the vehicle–vehicle control condition. The graph illustrates how combined doses of both drugs, which were ineffective independently, interacted to produce significant intake suppression.

SR141716 alone, produced any reliable effects on food intake. However, when given in combination, every dose of SR141716 potentiated the effects of all doses of naloxone. Significant intake suppression occurred with every combination of the two drugs, relative to the vehicle–vehicle, SR141716–vehicle or vehicle–naloxone conditions (Kirkham & Williams, 2001).

These data seem to indicate a synergistic interaction between the effects of opioid and cannabinoid receptor antagonists, and go a long way to support an important functional relationship between cannabinoid and opioid systems in the normal regulation of appetite. The dramatic nature of the combined effects of these drugs compared with their very weak independent effects are difficult to explain. However, given what we already know about opioids and feeding and CB1 agonist effects on eating microstructure, it is possible to argue that the suppression of feeding by combined opioid and cannabinoid receptor blockade may involve distinct actions on both appetitive (cannabinoid) and consummatory (opioid) aspects of eating motivation. If SR141716 acts to reduce the incentive value of food, while naloxone reduces food palatability once the animals begin to eat, we might predict that joint administration of the two drugs would act to delay the onset of feeding and, once initiated, lower the palatability of the test food and so reduce meal duration. Each effect alone may be insufficient to significantly affect intake, being obscured by the low resolution of measurement in our tests (i.e. by only measuring total intake after 1 h), but easily detectable when occurring contiguously. We are currently testing this hypothesis using observational analyses.

Whatever the behavioural alterations underlying these effects, our findings may have particular importance in the light of the proposed existence of a cannabinoid receptor subtype that is differentially linked to opioid systems (Welch & Eads, 1999). A further possibility is that these combinatorial effects reflect similar actions on ventral tegmental dopamine neurons. These neurons are under the inhibitory influence of GABAergic neurons in the ventral tegmental area. Endogenous opioids act to increase dopamine release in the accumbens by disinhibiting GABA neurons in the ventral tegmental area (Johnson & North, 1992). In addition, many of the neurons which express CB1 receptors are GABAergic, and the effect of CB1 agonists on these cells is again to reduce the release of GABA (Marsicano & Lutz, 1999). The marked effects of combined administration of opioid and cannabinoid receptor blockers might thus be explained in terms of enhanced GABAergic inhibition of mesolimbic dopamine activity. Obviously, a wide range of detailed pharmacological analyses are necessary to shed further light on these phenomena.

Another aspect of our research, which may shed further light on the mechanisms by which endocannabinoids influence appetite, involves mapping the brain sites mediating their actions. As we discussed earlier, CB1 receptors are expressed throughout the brain and the number of potential targets is extensive. However, one particular brain region has been increasingly linked to feeding and reward processes: the nucleus accumbens, and especially the shell sub-region of this nucleus (Kelley, 1999). We have obtained pilot data showing that several CB1 agonists, including anandamide, can induce feeding in this region (Kirkham & Williams, 1999). In our most recent experiments (TC Kirkham and CM Williams, unpublished results) we have also shown that hyperphagia can be obtained in freely-feeding rats by bilateral accumbens shell infusion of the endocannabinoid 2-arachidonoylglycerol. This latter finding represents the first demonstration of the ability of 2-arachidonoylglycerol to increase food intake, and provides compelling evidence for a natural role of this endocannabinoid in appetite regulation. Interestingly, short-term increases in food intake following 2-arachidonoylglycerol were almost wholly attributable to a reduction in meal latency, rather than increases in meal size. More experiments are obviously required to explore the brain sites sensitive to cannabinoid treatments, but the demonstration that

the accumbens is a sensitive target for endocannabinoid-induced feeding further strengthens the link between these neuromodulators and the reward processes outlined earlier.

Conclusion

The historical association between the effects of exogenous cannabinoids and appetite has provided scientists with an important lead to one of the possible physiological roles for the newly discovered endocannabinoid systems. While the potential of the exogenous cannabinoid, Δ^9-THC, to stimulate eating has been an accepted fact for many years, our review of the past literature shows that there are many questions about its action which remain unanswered. Work in our laboratory has shown that Δ^9-THC can induce a degree of overeating that matches that produced by other hyperphagic pharmacological manipulations. We have also demonstrated that these effects are due to interaction with central, endocannabinoid systems. In addition, we have now obtained very strong evidence that the endocannabinoids contribute to the normal mechanisms regulating appetite, through the demonstration that anandamide and 2-arachidonoylglycerol can also induce hyperphagia. Importantly, animals work harder to obtain food after CB1 stimulation, and eat sooner in a test, and more frequently than normal. Moreover, the changes to feeding behaviour induced by the endocannabinoids are entirely compatible with specific adjustments to eating motivation. Strengthening this hypothesis, we have seen that blockade of CB1 receptors reduces the willingness of laboratory animals to work for ingesta. Together with the other behavioural effects of exogenous or endogenous cannabinoids, these findings suggest that endocannabinoid systems are actively involved in the processes which drive us to eat.

In addition, we have presented evidence to suggest that endocannabinoid activity is not essential to the maintenance of ingestion, particularly ingestion maintained by palatability. However, we have also seen evidence of interactions between endogenous cannabinoids and the opioid systems which are intimately associated with orosensory reward. Such interactions suggest that, in addition to making food stimuli more salient, cannabinoid administration may also indirectly amplify the hedonic evaluation of foods. We have proposed that these combined actions on appetitive and consummatory aspects of feeding motivation may reflect modulation of classical, dopaminergic and opioidergic reward pathways. In line with this notion, we have demonstrated that the nucleus accumbens shell is a sensitive site for cannabinoid-induced hyperphagia. Overall, these different findings tend to confirm endocannabinoid involvement in the critical motivational processes underlying the stimulation of appetite. Experiments that we are currently conducting will attempt to establish the extent to which endocannabinoid activity contributes to the normal cyclicity of feeding and whether it may constitute a component of a normal 'hunger signal'.

Despite recent advances in cannabinoid pharmacology and a growing interest in the potential of cannabinoid-based therapies, current knowledge is severely handicapped by the lack of fundamental research into the behavioural actions of these substances. A willingness to accept essentially anecdotal accounts about cannabinoid actions, and to build hypotheses without access to even the most basic phenomenological data has slowed progress in this area considerably. However, we hope that our first steps in characterizing the behavioural actions of, and motivational processes served by endocannabinoids will soon give rise to both a better understanding of the neurochemical mechanisms regulating appetite and the promise of improved therapies for the treatment of disorders of eating and body weight.

The demonstration that cannabinoid receptor ligands can stimulate eating, and the possibility

that endocannabinoids could play an important role in the processes which give rise to hunger or appetite, may also help redress the current outlook on eating as merely a process to be limited. We hope that our research will contribute to a resurgence of interest in the biopsychological processes underlying so much of our individual and cultural approaches to food and eating. Our innate responsiveness to food stimuli and our capacity to overconsume are likely to be understood and managed more easily through analysis of the mechanisms that provoke appetite, rather than by pursuing yet more putative satiety factors. There are many more experiments to do, and many more avenues to follow. But we anticipate that future research will only consolidate our view of endocannabinoid systems as essential components of appetite regulation.

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References

Abel EL (1971) Effects of marijuana on the solution of anagrams, memory and appetite. *Nature* **231**, 260–261.

Abel EL (1975) Cannabis: effects on hunger and thirst. *Behavioral Biology* **15**, 255–281.

Abrahamov A, Abrahamov A & Mechoulam R (1995) An efficient new cannabinoid antiemetic in pediatric oncology. *Life Sciences* **56**, 2097–2102.

Arnone M, Maruani J, Chaperon F, Thiebot MH, Poncelet M, Soubrié P & LeFur G (1997) Selective inhibition of sucrose and ethanol intake by SR141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology* **132**, 104–106.

Beal JE, Olson R, Laubenstein L, Morales JO, Bellman P, Yangco B, Lefkowitz L, Plasse TF & Shepard KV (1995) Dronabinol as a treatment for anorexia associated with weight loss in patients with AIDS. *Journal of Pain & Symptom Management* **10**, 89–97.

Berridge K (1991) Modulation of taste affect by hunger, energy satiety, and sensory-specific satiety in the rat. *Appetite* **16**, 103–120.

Berridge K (2000) Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. *Neuroscience and Biobehavioral Reviews* **24**, 173–198.

Bidaut-Russell M & Howlett AC (1991) Cannabinoid receptor regulated cAMP accumulation in the rat striatum. *Journal of Neurochemistry* **57**, 1769–1773.

Bivens CLM, Thomas WJ & Stanley BG (1998) Similar feeding patterns are induced by perifornical neuropeptide Y injection and by food deprivation. *Brain Research* **782**, 271–280.

Breivogel CS & Childers SR (1998) The functional neuroanatomy of brain cannabinoid receptors. *Neurobiology of Disease* **5**, 417–431.

Breivogel CS, Sim LJ & Childers SR (1997) Regional differences in cannabinoid receptor/G-protein coupling in rat brain. *Journal of Pharmacology and Experimental Therapeutics* **282**, 1632–1642.

Brown JE, Kassouny M & Cross JK (1977) Kinetic studies of food intake and sucrose solution preference by rats treated with low doses of delta-9-tetrahydrocannabinol. *Behavioral Biology* **20** 104–110.

Burstein SH, Young JK & Wright GE (1995) Relationships between eicosanoids and cannabinoids: are eicosanoids cannabimimetic agents. *Biochemical Pharmacology* **50**, 1735–1742.

Cabeza de Vaca S & Carr KD (1998) Food restriction enhances the central rewarding effect of abused drugs. *Journal of Neuroscience* **18**, 7502–7510.

Cat LK & Coleman RL (1994) Treatment for HIV wasting syndrome. *Annals of Pharmacotherapy* **28**, 595–597.

Colombo G, Agabio R, Diaz G, Lobina C, Reali R & Gessa GL (1998) Appetite suppression and weight loss after the cannabinoid antagonist SR141716. *Life Sciences* **63**, PL113–PL117.

Clark JT, Kalra PS, Crowley WR & Kalra SP (1984) Neuropeptide Y and human pancreatic polypeptide stimulate feeding behaviour in rats. *Endocrinology* **115**, 427–429.

Clifton PJ (2000) Meal patterning in rodents: psychopharmacological and neuroanatomical studies. *Neuroscience and Biobehavioral Reviews* **24**, 213–222.

Cooper SJ & Clifton PJ (1996) *Drug Receptor Subtypes and Ingestive Behaviour*. London: Academic Press.

Cooper SJ & Kirkham TC (1993) Opioid mechanisms in the control of food consumption and taste preferences. In *Handbook of Experimental Pharmacology*, vol. 104, part II, pp. 239–263 [A Herz, H Akil and EJ Simon, editors]. Berlin: Springer–Verlag.

Crawley JN, Corwin RL, Robinson JK, Felder CC, Devane WA & Axelrod JA (1993) Anandamide, an endogenous ligand of the cannabinoid receptor, induces hypomotility and hypothermia in vivo in rodents. *Pharmacology Biochemistry and Behavior* **46**, 967–972.

De Petrocellis L, Melck D, Bisogno T, Milone A & Di Marzo V (1999) Finding of the endocannabinoid signalling system in Hydra, a very primitive organism: possible role in the feeding response. *Neuroscience* **92**, 377–387.

Devane WA, Dysarz FA, Johnson MR, Melvin LS & Howlett AC (1988) Determination and characterisation of a cannabinoid receptor in rat brain. *Molecular Pharmacology* **34**, 605–613.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A & Mechoulam R (1992) Isolation of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946–1949.

Dewey WL (1986) Cannabinoid pharmacology. *Pharmacological Reviews* **38**, 151–178.

DiMarzo V, Bifulco M & De Petrocellis L (2000) Endocannabinoids and multiple sclerosis: a blessing from the 'inner bliss'? *Trends in Pharmacological Sciences* **21**, 195–197.

Di Marzo V & Deutsch DG (1998) Biochemistry of endogenous ligands of cannabinoid receptors. *Neurobiology of Disease* **5**, 386–404.

Di Marzo V, Melck D, Bisogno T & De Petrocellis L (1998) Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. *Trends in Neurosciences* **21**, 521–528.

Drewnowski A & Grinker JA (1978) Food and water intake, meal patterns and activity of obese and lean Zucker rats following chronic and acute treatment with Δ^9-tetrahydrocannabinol. *Pharmacology Biochemistry and Behavior* **9**, 619–630.

Edwards CM, Abusnana S, Sunter D, Murphy KG, Ghatei MA & Bloom SR (1999) The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. *Journal of Endocrinology* **160**, R7–R12.

Fimiani C, Liberty T, Aquirre AJ, Amin I, Ali N & Stefano GB (1999) Opiate, cannabinoid, and eicosanoid signalling converges on common intracellular pathways nitric oxide coupling. *Prostaglandins & Other Lipid Mediators* **57**, 23–34.

Foltin RW, Brady JV & Fischman MW (1986) Behavioral analysis of marijuana effects on food intake in humans. *Pharmacology Biochemistry and Behavior* **25**, 577–582.

Foltin RW, Fischman MW & Byrne MF (1988) Effects of smoked marijuana on food intake and body weight of humans living in a residential laboratory. *Appetite* **11**, 1–14.

Fuentes JA, Ruiz Gayo M, Manzanares J, Vela G, Reche I & Corchero J (1999) Cannabinoids as potential new analgesics. *Life Sciences* **65**, 675–685.

Gallate JE & McGregor IS (1999) The motivation for beer in rats: effects of ritanserin, naloxone and SR141716. *Psychopharmacology* **142**, 302–308.

Gallate JE, Saharov T, Mallet PE & McGregor IS (1999) Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. *European Journal of Pharmacology* **370**, 233–240.

Gaoni Y & Mechoulam R (1964) Isolation, structure and partial synthesis of an active constituent of hashish. *Journal of the American Chemical Society* **86**, 1646.

Gardner EL (1992) Cannabinoid interaction with brain reward systems – the neurobiological basis of cannabinoid abuse. In *Marijuana/Cannabinoids: Neurobiology and Neurophysiology*, pp. 275–335 [LL Murphy and A Bartke, editors]. New York, NY: CRC Press.

Gardner EL, Paredes W, Smith D, Donner A, Milling C, Cohen D & Morrison D (1988) Facilitation of brain stimulation reward by Δ-9-tetrahydrocannabinol. *Psychopharmacology* **96**, 142–144.

Gardner EL & Vorel SR (1998) Cannabinoid transmission and reward-related events. *Neurobiology of Disease* **5**, 502–533.

Glass M, Dragunow M & Faull RLM (1997) Cannabinoid receptors in the human brain: A detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* **77**, 299–318.

Glick SD & Milloy S (1972) Increased and decreased eating following THC administration. *Psychonomic Science* **29**, 6.

Gorter RW (1999) Cancer cachexia and cannabinoids. *Forsch Komplemetarmed* **6**, Suppl. 3, 21–22.

Gosnell BA, Krahn DD & Majchrzak MJ (1990) The effects of morphine on diet selection are dependent on base-line diet preferences. *Pharmacology Biochemistry and Behavior* **37**, 207–212.

Graceffo TJ & Robinson JK (1998) Delta-9-tetrahydrocannabinol (THC) fails to stimulate consumption of a highly palatable food in the rat. *Life Sciences* **62**, 85–88.

Gralla RJ (1999) Cannabinoids and the control of chemotherapy-induced nausea and vomiting. In *Marihuana and Medicine*, pp. 599–610 [GG Nahas, KM Sutin, DJ Harvey and S Agurell, editors]. Totowa, NJ: Humana Press.

Greenberg I, Kuehnle J, Mendelson JH & Bernstein JG (1976) Effects of marijuana use on body weight and energy intake in humans. *Psychopharmacology* **49**, 79–84.

Haines L & Green W (1970) Marijuana use patterns. *British Journal of Addiction* **65**, 347–362.

Halikas JA, Goodwin DW & Guze SB (1971) Marijuana effects. A survey of regular users. *Journal of the American Medical Association* **217**, 692–694.

Hampson AJ, Grimaldi M, Lolic M, Wink D, Rosenthal R & Axelrod J (2000) Neuroprotective antioxidants from marijuana. *Annals of the New York Academy of Sciences* **899**, 274–282.

Hao S, AvrahamY, Mechoulam R & Berry EM (2000) Low dose anandamide affects food intake, cognitive function, neurotransmitter and corticosterone levels in diet-restricted mice. *European Journal of Pharmacology* **392**, 147–156.

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, De Costa BR & Rice KC (1990) Cannabinoid receptor localisation in brain. *Proceedings of the National Academy of Sciences USA* **87**, 1932–1936.

Hoebel BG (1997) Neuroscience and appetitive behavior research: 25 years. *Appetite* **29**, 119–133.

Hollister LE (1971) Hunger and appetite after single doses of marijuana, alcohol and dextroamphetamine. *Clinical Pharmacology and Therapeutics* **12**, 45–49.

Hollister LE (1986) Health aspects of cannabis. *Pharmacological Reviews* **38**, 1–20.

Holst JJ (1999) Glucagon-like peptide 1 (GLP-1): an intestinal hormone, signalling nutritional abundance, with an unusual therapeutic potential. *Trends in Endocrinology and Metabolism* **10**, 229–235.

Ikemoto S & Panksepp J (1999) The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. *Brain Research Reviews* **31**, 6–41.

Johnson SW & North RA (1992) Opioids excite dopamine neurons by hyperpolarization of local interneurons. *Journal of Neuroscience* **12**, 483–488.

Kelley AE (1999) Functional specificity of ventral striatal compartments in appetitive behaviors. *Annals of the New York Academy of Sciences* **877**, 71–90.

Kirkham TC (1990) Enhanced anorectic potency of naloxone in rats sham feeding 30% sucrose: reversal by repeated naloxone administration. *Physiology and Behavior* **47**, 419–426.

Kirkham TC & Cooper SJ (1988) Naloxone attenuation of sham feeding is modified by manipulation of sucrose concentration. *Physiology and Behavior* **44**, 491–494.

Kirkham TC & Cooper SJ (1991) Opioid peptides in relation to the treatment of obesity and bulimia. In *Peptides: A Target for New Drug Development*, pp. 28–44 [SR Bloom and G Burnstock, editors]. London: IBC.

Kirkham TC & Williams CM (1998) The cannabinoid receptor antagonist SR141716 fails to suppress sucrose sham feeding. *Journal of Psychopharmacology* **12**, Suppl. A, 37.

Kirkham TC & Williams CM (1999) Central administration of anandamide induces hyperphagia in satiated rats. *Society for Neuroscience Abstracts* **25**, 1885.

Kirkham TC & Williams CM (2001) Synergistic effects of opioid and cannabinoid antagonists on food intake. *Psychopharmacology* **153**, 267–270.

Ledent C, Valverde O, Cossu G, Petite F, Aubert J-F, Baslet F, Bohme GA, Imperato A, Pedrazzini T, Roques BP, Vassert G, Fratta W & Parmentier M (1999) Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* **283**, 401–404.

Leventhal L, Kirkham TC, Cole JL, Bodnar RJ (1995) Selective actions of central mu and kappa opioid antagonists upon sucrose intake in sham-feeding rats. *Brain Research* **685**, 205–210.

McLaughlin CL, Baile CA & Bender PE (1979) Cannabinols and feeding in sheep. *Psychopharmacology* **64**, 321–323.

Manning FJ, McDonough JH, Elsmore TF, Saller C & Sodetz FJ (1971) Inhibition of normal growth by chronic administration of Δ9-tetrahydrocannabinol. *Science* **174**, 424–426.

Marsicano G & Lutz B (1999) Expression of the cannabinoid receptor CB1 in distinct neuronal subpopulations in the adult mouse forebrain. *European Journal of Neuroscience* **11**, 4213–4225.

Mascia MS, Obinu MC, Ledent C, Parmentier M, Bohme GA, Imperato A & Fratta W (1999) Lack of morphine-induced dopamine release in the nucleus accumbens of cannabinoid CB1 receptor knockout mice. *European Journal of Pharmacology* **383**, R1–R2.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC & Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346**, 561–564.

Mattes RD, Engelman K, Shaw LM & Elsohly MA (1994) Cannabinoids and appetite stimulation. *Pharmacology Biochemistry and Behavior* **49**, 187–195.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J & Vogel Z (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochemical Pharmacology* **50**, 83–90.

Mechoulam R, Shani A, Edery H & Grunfield Y (1970) Chemical basis of hashish activity. *Science* **169**, 611.

Mela DJ & Rogers PJ (1998) *Food Eating and Obesity*. London: Chapman and Hall.

Munro S, Thomas KL & Abu-Shaar M (1993) Molecular characterisation of a peripheral receptor for cannabinoids. *Nature* **365**, 61–65.

Nahas GG, Sutin KM, Harvey DJ & Agurell S (1999) *Marihuana and Medicine*. Totowa, NJ: Humana Press.

Onaivi ES, Chakrabarti A & Chaudhuri G (1996) Cannabinoid receptor genes. *Progress in Neurobiology* **48**, 275–305.

Pertwee RG (1995) *Cannabinoid Receptors*. London: Academic Press.

Pertwee RG (2000) Neuropharmacology and therapeutic potential of cannabinoids. *Addiction Biology* **5**, 37–46.

Piomelli D, Beltramo M, Giuffrida A & Stella N (1998) Endogenous cannabinoid signaling. *Neurobiology of Disease* **5**, 462–473.

Plasse TF, Gorter RW, Krasnow SH, Lane M, Shepard KY & Wadleigh RG (1991) Recent clinical experience with dronabinol. *Pharmacology Biochemistry and Behavior* **40**, 695–700.

Regelson W, Butler JR & Schultz J (1976) Delta-9-tetrahydrocannabinol as an effective antidepressant and appetite-stimulating agent in advanced cancer patients. In *The Pharmacology of Marijuana*, pp. 763–776 [MC Braude and S Szara, editors]. New York, NY: Raven Press.

Reggio PH (1999) Cannabinoid receptors. *Tocris Reviews* **10**, 1–5.

Rodriguez de Fonseca F, Martin Calderon, JL, Mechoulam R & Navarro M (1994) Repeated stimulation of D1 dopamine receptors enhances (2)-11-hydroxy-delta-8-tetrahydrocannabinol-dimethyl-heptyl-induced catalepsy in male rats. *Neuroreport* **5**, 761–765.

Rodriguez de Fonseca F, Roberts AJ, Bilbao A, Koob GF & Navarro M (1999) Cannabinoid receptor antagonist SR141716 decreases operant ethanol self administration in rats exposed to ethanol-vapor chambers. *Acta Pharmacologica Sinica* **20**, 1109–1114.

Sacks N, Hutcheson JR, Watts JM & Webb RE (1990) Case report: the effect of tetrahydrocannabinol on food intake during chemotherapy. *Journal of the American College of Nutrition* **9**, 630–632.

Sanudo-Pena MC, Patrick SL, Patrick RL & Walker JM (1996) Effects of intranigral cannabinoids on rotational behaviour in rats: Interaction with the dopaminergic system. *Neuroscience Letters* **206**, 21–24.

Schwartz MW, Woods SC, Porte D Jr, Seeley RJ & Baskin DG (2000) Central nervous system control of food intake. *Nature* **404**, 661–671

Simiand J, Keane M, Keane PE & Soubrie P (1998) SR141716, a CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset. *Behavioural Pharmacology* **9**, 179–181.

Sjoden P-O, Jarbe TUC & Henriksson BG (1973) Influence of tetrahydrocannabinols (Δ-8-THC and Δ-9-THC) on body weight, food and water intake in rats. *Pharmacology Biochemistry and Behavior* **1**, 395–399.

Soderstrom K, Leid M, Moore FL & Murray TF (2000) Behavioral, pharmacological, and molecular characterization of an amphibian cannabinoid receptor. *Journal of Neurochemistry* **75**, 413–423.

Sofia RD & Barry H (1974) Acute and chronic effects of Δ-9-tetrahydrocannabinol on food intake by rats. *Psychopharmacologia* **39**, 213–222.

Souilhac J, Poncelet M, Rinaldi-Carmona M, Le Fur G & Soubrie P (1995) Intrastriatal injection of cannabinoid receptor agonists induced turning behavior in mice. *Pharmacology Biochemistry and Behavior* **51**, 3–7.

Spanagel R & Weiss F (1999) The dopamine hypothesis of reward: past and current status. *Trends in Neurosciences* **22**, 521–527.

Stella N, Schweitzer P & Piomelli D (1997) A second endogenous cannabinoid that modulates long-term potentiation. *Nature* **388**, 773–778.

Stratford TR, Swanson CJ & Kelley A (1998) Specific changes in food intake elicited by blockade or activation of glutamate receptors in the nucleus accumbens shell. *Behavioral Brain Research* **93**, 43–50.

Stuwe M, Kaempfer SH, Geiger CJ, Pavia AT, Plasse TF, Shepard KV, Ries K & Evans TG (1993) Effect of dronabinol on nutritional status in HIV infection. *Annals of Pharmacotherapy* **27**, 827–831.

Tanda G, Pontieri FE & DiChiara G (1997) Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common μ_1 opioid receptor mechanism. *Science* **276**, 2048–2050.

Tart C (1970) Marijuana intoxication: common experiences. *Nature* **226**, 701–704.

Trojniar W & Wise RA (1991) Facilitory effect of Δ-9-tetrahydrocannabinol on hypothalamically-induced feeding. *Psychopharmacology* **103**, 172–176.

Volicer L (1997) Dronabinol may help behaviour problems in Alzheimers disease. *American Family Physician* **55**, 1338.

Weingarten HP & Watson SD (1982) Sham feeding as a procedure for assessing the influence of diet palatability on food intake. *Physiology and Behavior* **28**, 401–407.

Welch SP & Eads EM (1999) Synergistic interactions of endogenous opioids and cannabinoid systems. *Brain Research* **848**, 183–190.

Williams CM, Rogers PJ & Kirkham TC (1998) Hyperphagia in pre-fed rats following oral Δ-9-THC. *Physiology and Behavior* **65**, 343–346.

Williams CM & Kirkham TC (1999*a*) Anandamide induces overeating: mediation by central cannabinoid receptors. *Psychopharmacology* **143**, 315–317.

Williams CM & Kirkham TC (1999*b*). Observational analysis of Δ9-THC effects on feeding microstructure in rats. *Journal of Psychopharmacology* **13** (3, Suppl. A), 20.

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Cannabinoids and multiple sclerosis

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Abstract

There is a growing amount of evidence to suggest that cannabis and individual cannabinoids may be effective in suppressing certain symptoms of multiple sclerosis and spinal cord injury, including spasticity and pain. Anecdotal evidence is to be found in newspaper reports and also in responses to questionnaires. Clinical evidence comes from trials, albeit with rather small numbers of patients. These trials have shown that cannabis, Δ^9-tetrahydrocannabinol, and nabilone can produce objective and/or subjective relief from spasticity, pain, tremor, and nocturia in patients with multiple sclerosis (8 trials) or spinal cord injury (1 trial). The clinical evidence is supported by results from experiments with animal models of multiple sclerosis. Some of these experiments, performed with mice with chronic relapsing experimental allergic encephalomyelitis (CREAE), have provided strong evidence that cannabinoid-induced reductions in tremor and spasticity are mediated by cannabinoid receptors, both CB_1 and CB_2. Endocannabinoid concentrations are elevated in the brains and spinal cords of CREAE mice with spasticity, and in line with this observation, spasticity exhibited by CREAE mice can be ameliorated by inhibitors of endocannabinoid membrane transport or enzymic hydrolysis. Research is now needed to establish whether increased endocannabinoid production occurs in multiple sclerosis. Future research should also be directed at obtaining more conclusive evidence about the efficacy of cannabis or individual cannabinoids against the signs and symptoms of these disorders, at devising better modes of administration for cannabinoids and at exploring strategies that maximize separation between the sought-after therapeutic effects and the unwanted effects of these drugs.

Keywords: Multiple sclerosis; Spinal cord injury; Pain; Cannabis; Tetrahydrocannabinol; Nabilone

Abbreviations: CREAE, chronic relapsing experimental allergic encephalomyelitis; EAE, experimental autoimmune encephalomyelitis; THC, tetrahydrocannabinol; TMEV, Theiler's murine encephalomyelitis virus.

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1. Introduction

Multiple sclerosis is a disorder of the nervous system in which the ability of neurons to conduct impulses becomes impaired through the loss of myelin, which normally forms the outer covering of many nerve fibres, and through axonal loss. These changes may result from inappropriate immune responses by patients. The nature of the resulting symptoms depends on where the demyelination and axonal loss have occurred. The signs and symptoms of multiple sclerosis fluctuate unpredictably, and tend to worsen with age. They can include painful muscle spasms, tremor, ataxia, weakness or paralysis, difficulty in speaking, constipation, and loss of bladder control. Some of these signs and symptoms can also be experienced by patients with spinal cord injury. This

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review summarizes anecdotal, clinical, and non-clinical evidence that cannabinoids have an important part to play in the clinical management of multiple sclerosis and spinal cord injury through an ability to suppress signs and symptoms of these disorders.

2. Anecdotal evidence

The idea that cannabinoids have the ability to suppress signs and symptoms of multiple sclerosis and spinal cord injury is in line with some traditional medical applications of cannabis. Thus, there are allusions in historical documents to the use of cannabis in ancient China, India, Greece, and Rome for easing the muscles of the limbs or for relieving muscle spasms, cramps, or rheumatic pains (see Mechoulam, 1986). Medical applications such as these were also recognized for cannabis by 19th century physicians such as W. B. O'Shaughnessy and J. R. Reynolds (see Mechoulam, 1986). More recently, particularly in the last 20 years, there have been many claims from multiple sclerosis patients about the benefits of self-medicating with cannabis. For example, Clare Hodges of the Alliance for Cannabis Therapeutics, who has multiple sclerosis, wrote in 1993:

> I was being prescribed a whole range of medicines. There were pills to stop me feeling sick. These made me clumsy and drowsy. There were pills to relieve bladder spasms but they made me feel sick and gave me blurred vision. There were pills to help me sleep but they made me anxious and were habit-forming,
>
> and then
>
> For about a year now, I have been regularly taking a small amount of cannabis resin—less than the size of half a pea—late at night. I used to smoke it...but I was worried that my children might see me smoking so now I eat it. After a short time, my body completely relaxes, which relieves my tension and spasms. During the day I have to use a catheter whenever I want to empty my bladder and, most notably, cannabis relieves the discomfort and difficulty I have controlling it. It has also stopped the nausea that kept me awake at night,
>
> and
>
> I don't often take enough to "get high". When I do, I'm sure the feeling of calm and euphoria does my spirits a lot of good, too.

Reports of this kind provoked the setting up in 1994 of a survey directed at more precisely establishing the claims that were being made about cannabis for multiple sclerosis and at identifying the most appropriate questions that should be addressed in any future clinical trials (Consroe et al., 1997). This questionnaire was distributed to multiple sclerosis patients who were thought to be self-medicating with cannabis, and replies were received anonymously from 57 men and 55 women (age range, 22–67 years). Of these, 59 were from the United States and 53 from the United Kingdom. Over 90% of those who were experiencing the following symptoms reported improvement after taking cannabis: spasticity at sleep onset (96.5%), pain in muscles (95.1%), spasticity when waking at night (93.2%), pain in the legs at night (92.3%), tremor of arms/head (90.7%), and depression (90.6%). The number of subjects reporting these symptoms were, respectively, 86, 61, 59, 52, 43, and 74. It was also reported that cannabis relieved a number of other symptoms. More specifically, of those who were experiencing particular symptoms, the percentage of those reporting improvement in response to cannabis was 81–90% for anxiety, for spasticity when waking in the morning or when walking, and for tingling in face/arms/legs/trunk; 71–80% for numbness of chest/stomach, face pain, weight loss, and leg weakness; 61–70% for tiredness, urinary urgency, double vision, and sexual dysfunction; 51–60% for ability to walk, urinary hesitancy, vision dimness, defaecation urgency, balance, urinary incontinence, and slurred speech; 44% for faecal incontinence; 32% for memory loss; and 30% for constipation. It was also claimed that cannabis was taken at all times of day (more in the evening than at other times), 2–3 times per day (mean, 2.7 times) and 5–6 days per week (mean, 5.6 days), and that the cannabis was usually smoked. Because this survey targeted multiple sclerosis patients who self-medicate with cannabis, the data it generated cannot be used to predict the proportion of all with multiple sclerosis who might benefit from cannabis or individual cannabinoids. Multiple sclerosis patients tend to exhibit a high rate of positive responding to placebo treatments. Even so, the high proportion of those who claim cannabis to relieve spasticity, tremor, and pain is impressive. Indeed, as it is also in line both with evidence from animal experiments and with the limited amount of clinical data that has been gathered already, it clearly warrants further clinical investigation.

Claims that cannabis can reduce spasticity and pain are also to be found in two surveys of patients with spinal cord injury. In one of these surveys, conducted with 10 patients, 5 out of 8 patients with spasticity reported improvement in response to cannabis self-medication (Dunn & Davis, 1974). Cannabis-induced improvement was also noted by five out of nine patients with headache and by four out of nine patients with phantom limb pain. However, one of the patients reported cannabis to increase phantom limb pain. Bladder spasms decreased after cannabis in one patient, but increased after cannabis in another patient, and two patients noted increased urinary retention after cannabis. In the other survey, 21 out of 24 patients with spinal cord injury claimed that cannabis self-medication decreased spasticity (Malec et al., 1982). More recently, Schnelle et al. (1999) carried out a survey directed at determining the incidence and nature of the medical use of cannabis and cannabinoids in Germany, Austria, and Switzerland. They reported that only 5 of the 128 patients surveyed took Δ^9-tetrahydrocannabinol (THC, Marinol®) by prescription and that the remainder used natural cannabis products. Among a range of claimed

therapeutic targets were multiple sclerosis, back pain, spasticity, and spinal cord injury.

3. Clinical evidence

The clinical evidence comes from eight clinical trials performed with a rather small number of multiple sclerosis patients and from a study of one patient with spinal cord injury. Five of these investigations were carried out with orally administered Δ^9-THC, the results obtained suggesting that this treatment can reduce the intensity of several signs and symptoms of multiple sclerosis or spinal cord injury (Table 1). In particular, objective testing has provided evidence that Δ^9-THC can decrease spasticity, rigidity, and tremor and can improve walking ability, performance in a handwriting test, and bladder control. There were also claims from the patients who took part in one or another of these trials that oral Δ^9-THC improves spasticity, tremor, mobility, and quality of sleep; relieves pain; and induces a sense of well-being. In one double-blind trial with a single patient with spinal cord injury in which the effects of Δ^9-THC and codeine were compared, it was found that oral Δ^9-THC (5 mg) reduced pain and spasticity, whereas oral codeine (50 mg) had only an analgesic effect (Maurer et al., 1990).

Three clinical trials have been carried out with orally administered nabilone and two with inhaled or orally administered cannabis (Table 1). These yielded results that are broadly in line with the findings obtained in the clinical trials with oral Δ^9-THC. They also showed that smoked cannabis could reduce ataxia in one multiple sclerosis patient (Meinck et al., 1989) and could decrease nystagmus amplitude and improve visual acuity in another (Schon et al., 1999). In some instances, improvements reported by patients in response to Δ^9-THC have not been detectable in objective tests (Table 1). For example, when Ungerleider et al. (1987) gave Δ^9-THC (7.5 mg p.o.) to 8 multiple sclerosis patients with significant spasticity, this treatment decreased subjective levels of spasticity without improving performance in objective functional tests in which assessments were made of limb weakness, limb spasticity, limb coordination, gait impairment, and reflexes. Possible explanations for this discrepancy are that the objective tests were insufficiently sensitive or that these tests were applied at a single time point, whereas self-rating took place over a 24-hr period (Ungerleider et al., 1987).

The cannabinoid receptor agonist nabilone is licensed in the United Kingdom for the suppression of nausea and vomiting provoked by anticancer drugs. However, it is also permissible to prescribe nabilone in the United Kingdom for other purposes on a "named patient basis," and Dr. William Notcutt is one British pain specialist who offers nabilone to patients experiencing chronic pain that has been found to be untreatable by conventional therapy. Notcutt et al. (1999) have reported the clinical outcome of giving nabilone orally to 60 patients. These included 16 with advanced multiple sclerosis, of which, 6 experienced analgesia, muscle relaxation, and/or sleep improvement after nabilone. However, the other 10 multiple sclerosis patients obtained no useful benefit from the drug. Notcutt et al. (1999) have also found that a large proportion of the patients they have treated with nabilone experienced drowsiness and dysphoria of sufficient severity to cause many to discontinue the drug, in spite of obtaining a benefit. The intrinsic properties of nabilone, the difficulty of controlling its bioavailability (see next paragraph), and the inability to titrate nabilone against fluctuations of pain through the day were all cited as possible reasons for this problem.

Orally administered cannabinoids seem to undergo somewhat variable absorption. For example, Clifford (1983) found that the oral dose of Δ^9-THC required to improve performance in a handwriting test was 5 mg for one multiple sclerosis patient, but 15 mg for a second patient, and that both 5 and 10 mg were ineffective in the second patient. In another investigation (Schon et al., 1999), it was found that whilst smoked cannabis reduced nystagmus amplitude in a multiple sclerosis patient, no improvement was detectable in response to orally administered cannabis capsules or, indeed, to oral nabilone at up to 6 mg per day. There is also evidence that oral Δ^9-THC has a rather narrow therapeutic window. This comes from experiments in which 13 multiple sclerosis patients with significant spasticity were subjected to escalating daily doses of oral Δ^9-THC in the range 2.5–15 mg (Ungerleider et al., 1987). Subjective levels of spasticity reported by these patients decreased after 7.5-, 10-, or 15-mg Δ^9-THC. No improvement was experienced after Δ^9-THC at doses of 2.5 or 5 mg, whilst 3 of 4 patients receiving 10-mg Δ^9-THC reported intolerable side effects. It follows that one area for future clinical research is the development of cannabinoid formulations and modes of administration that produce more reliable cannabinoid absorption than has hitherto been achievable, at least by the oral route. Possible solutions are to develop improved oral formulations or to use other routes for cannabinoid delivery, for example, administration by rectal suppository (Brenneisen et al., 1996), by aerosol/vapour inhalation, by injection, by skin patch, or by the sublingual or intrathecal route, all modes of administration that avoid first-pass metabolism of the absorbed drug. Some success has been achieved already in Phase I studies with a sublingual cannabinoid spray (Whittle et al., 2001). The emergence of better modes of cannabinoid administration should be facilitated by the development of a centrally active water-soluble cannabinoid (Pertwee et al., 2000).

The absorption difficulties that seem to be associated with the oral administration of Δ^9-THC may account for anecdotal claims by multiple sclerosis patients that cannabis is superior to Δ^9-THC as a medicine, as the comparison is usually between oral Δ^9-THC (slow, unreliable absorption followed by hepatic first-pass metabolism to active and inactive metabolites) and smoked cannabis (faster, more reliable absorption, with no first-pass metabolism). How-

Table 1
Effects of cannabis or single cannabinoids on signs and symptoms of multiple sclerosis and spinal cord injury

Signs and symptoms	Design	Treatment	Measured effect	Reference
Spasticity related to multiple sclerosis in 9 patients naïve to cannabis	Double-blind, placebo	THC (p.o.) 5 or 10 mg	Objective "spasticity score" improved significantly ($P<0.005$ vs. placebo); feel "better able to walk" (3 patients); feel "high" (1 patient; 10 mg)	Petro & Ellenberger, 1981
		Placebo	Objective "spasticity score" improved (1 patient); feel " high" (1 patient)	
Disabling tremor and mild ataxia related to multiple sclerosis in 1 cannabis-experienced male patient (aged 30)	Single-blind, placebo	THC (p.o.) 5 mg	Mild subjective improvement in tremor and sense of well-being; performance in handwriting test improved; long-lasting decrease in head and neck tremor; little change in mild hand ataxia (finger-nose-finger testing); mild "high"	Clifford, 1983
		Placebo	No improvement; "high" sensation	
Disabling tremor and other signs related to multiple sclerosis in 1 female patient (aged 30)	Single-blind, placebo	THC (p.o.) 15 mg	Mild subjective improvement in tremor and sense of well-being; long-lasting improved performance in handwriting test (5 and 10 mg ineffective); other signs of motor dysfunction were not alleviated; "high" sensation	
		Placebo	No improvement	
Six other multiple sclerosis patients with disabling tremor and ataxia, some cannabis-experienced (aged 21–49)	Single-blind, placebo	THC (p.o.) 5–15 mg	Mild subjective improvement in tremor and sense of well-being in 5 of the patients; no objective improvement; "high" sensation	
Spasticity, limb weakness, hyperactive reflexes, and impaired coordination and gait related to multiple sclerosis in 5 male and 8 female patients aged 26–64 (9 of these patients were cannabis-experienced)[2]	Double-blind, placebo, cross-over	THC (p.o.) 7.5 mg[1]	Subjective improvement in spasticity; performance in objective function tests not improved; tolerable side effects (10 mg was intolerable to some patients; 2.5 and 5 mg ineffective)	Ungerleider et al., 1987
		Placebo	No improvement; THC-like subjective effects in 5 patients	
Spasticity and pain due to spinal cord injury in 1 male patient (aged 28)	Double-blind, placebo	THC (p.o.) 5 mg[3]	Marked reductions in pain and self-rated spasticity; improvements in bladder control, quality of sleep, mood, and ability to concentrate on intellectual work next day	Maurer et al., 1990
Multiple dysmorphy and cervical myelopathy with progressive spastic tetraparesis in 1 male patient (aged 48) and multiple sclerosis and light cervical myelopathy in another male patient (aged 64)	Open-label	THC (p.o.) 10 or 15 mg or THC hemisuccinate (rectal[4])	THC and THC hemisuccinate improved walking ability and passive mobility (Ashworth Scale); they also reduced rigidity and produced slight pain relief in the younger patient; temporary deterioration in ability to concentrate and in mood after	Brenneisen et al., 1996

Table 1 (*continued*)

Signs and symptoms	Design	Treatment	Measured effect	Reference
			THC (older patient); neither formulation affected miction frequency, blood pressure, heart rate, or body temperature	
Muscle spasms, nocturia, and other signs related to multiple sclerosis in 1 male patient (aged 45)	Double-blind, placebo, cross-over	Nabilone (p.o.) 1 mg (every second day)	Subjective improvement in painful muscle spasms, mood, and well-being; reduction in frequency of nocturia	Martyn et al., 1995
Severe intractable pain in both legs of 1 male patient caused by multiple sclerosis	Open-label?	Nabilone (p.o.) 1 mg (twice daily)	Pain relieved completely[5,6]	Hamann & di Vadi, 1999
Signs of multiple sclerosis with oscillopsia associated with prominent pendular nystagmus in 1 male patient (aged 52)	Placebo	Cannabis or tobacco[7] (inhaled)	Nystagmus amplitude reduced and visual acuity improved; nystagmus frequency unchanged	Schon et al., 1999
	Open-label?	Cannabis oil capsules[8] or nabilone[9] (p.o.)	No discernible benefit	
Spastic tetraparesis, limb and gait ataxia, intention tremor, and other signs related to multiple sclerosis in 1 cannabis-experienced male patient (aged 30)	Open-label	Cannabis (inhaled)	Objective testing showed marked reduction in spasticity, hand and finger action tremor (intention tremor) almost abolished, ataxia reduced (finger-nose test), and mobility improved	Meinck et al., 1989

[1] This dose was given to 8 of the 13 patients.
[2] History of intolerable side-effects from antispasticity drugs, including baclofen, dantrolene, and diazepam.
[3] THC and placebo were taken with baclofen (40 mg) and clonazepam (1 mg).
[4] Dose of THC hemisuccinate equivalent to 2.5- or 5-mg THC.
[5] Trials with a variety of unspecified antineuropathic and antinociceptive treatments were unsuccessful.
[6] Analgesia not reversed by naloxone (200 μg i.v.).
[7] Nystagmus unaffected by tobacco-only cigarettes.
[8] Up to 8 cannabis oil capsules per day, with each capsule equivalent to about 5-mg Δ^9-THC.
[9] Up to 6-mg nabilone per day.

ever, it is unlikely that smoked cannabis would itself ever be acceptable for the clinic. Thus, because of the tars and gases produced during the combustion process, cannabis smoke is toxic to airway tissue, and probably carcinogenic (Fung et al., 1999; Hollister, 1986; Sherman et al., 1991).

Some signs of multiple sclerosis or spinal cord injury may be worsened by cannabinoids. Thus, in a double-blind randomized placebo-controlled study, Greenberg et al. (1994) found that although cannabis cigarettes (1.54% Δ^9-THC), smoked on one occasion by ten 21- to 55-year-old multiple sclerosis patients with spasticity and gait dysfunction, produced a subjective feeling of clinical improvement, they also caused a subtle impairment of posture and balance, as measured by "dynamic posturography." The posture and balance of 10 matched healthy subjects was also impaired. There have been other reports that cannabis can impair postural control in healthy subjects (see Paton & Pertwee, 1973), and it is well documented that cannabinoids cause dogs to weave to and fro whilst remaining fixed in one spot (the basis of the "static ataxia" bioassay for cannabinoids) (Martin et al., 1995; Razdan, 1986).

The clinical reports that cannabinoids can reduce pain caused by multiple sclerosis or spinal cord injury are supported by evidence from other clinical investigations that intramuscular injection of the cannabinoid receptor agonist L-nantradol is effective against acute postoperative pain (Jain et al., 1981), that Δ^9-THC (10 mg p.o.) can relieve cancer pain (Noyes et al., 1975a, 1975b), and that oral cannabis had a morphine-sparing effect in a patient suffering from severe chronic abdominal pain (Holdcroft et al., 1997).

4. Non-clinical evidence

Results obtained with animal models of multiple sclerosis provide strong support for the claimed benefits of cannabinoids for this disorder. More specifically, data from experiments with rats and guinea-pigs (Lyman et al., 1989;

Wirguin et al., 1994) have indicated that the cannabinoid receptor agonists Δ^8- and Δ^9-THC decrease signs of experimental autoimmune encephalomyelitis (EAE). In these experiments, EAE was induced in Lewis rats, Sabra outbred rats, or strain 13 guinea-pigs by inoculation with *Myobacterium tuberculosis* in combination with Freund's complete adjuvant and guinea-pig myelin basic protein or homogenates of spinal cord or bovine white matter (and sometimes also with *Bordetella pertussis* vaccine). The animals were then observed for up to 21 days. Δ^8-THC, Δ^9-THC, or vehicle were given once daily, the first administration being made between 1 and 9 days after inoculation. The guinea-pigs received daily intraperitoneal injections of 5-mg Δ^9-THC (Lyman et al., 1989) and the rats, oral administrations of 5-mg/kg Δ^9-THC (Lyman et al., 1989) or 40-mg/kg Δ^8-THC (Wirguin et al., 1994). Following these drug treatments, the clinical signs of EAE, which can progress from tail flaccidity (rats) and generalized atonia to death via ataxia, paraparesis, incontinence, paraplegia, and quadriplegia/moribundity, were delayed in onset and reduced in intensity. Lyman et al. (1989) also found Δ^9-THC to decrease histological signs of EAE inflammation in rat and guinea-pig spinal cord. Dexanabinol (HU-211), a synthetic cannabinoid with *N*-methyl-D-aspartate-blocking and antioxidant properties that does not share the ability of Δ^8- or Δ^9-THC to act though CB_1 or CB_2 receptors, has also been found to decrease signs of EAE in rats (Achiron et al., 2000). Possible mechanisms underlying this effect of dexanabinol are inhibition of tumour necrosis factor-α release and neuroprotection through scavenging of free radicals.

Baker et al. (2000) have investigated the part played by cannabinoid receptors in cannabinoid-induced suppression of the spasticity and tremor of mice with chronic relapsing experimental allergic encephalomyelitis (CREAE). This is an autoimmune model of multiple sclerosis that is set up by injecting Biozzi ABH mice subcutaneously with an emulsion of mouse spinal cord homogenate in Freund's complete adjuvant on days 0 and 7. This treatment induces sensitization to myelin antigens, which leads to demyelination and axonal loss in the CNS and, hence, to the production of relapsing-remitting episodes of hind-limb spasticity and unilateral or bilateral forelimb and hind-limb tremor. It was found that limb spasticity and tremor exhibited by CREAE mice could be readily suppressed by the cannabinoid receptor agonists *R*-(+)-WIN-55212 (1, 2.5, or 5 mg/kg i.p.) and Δ^9-THC (10 mg/kg i.v.). These effects seemed to be cannabinoid receptor-mediated since limb spasticity was not reduced by the *S*-(−)-enantiomer of WIN-55212 or by (−)-cannabidiol, both of which lack significant cannabinoid receptor affinity. Unexpectedly, evidence was obtained that suppression of limb spasticity and/or tremor could be mediated not just by CB_1 receptors, but also by CB_2 receptors. Thus, Baker et al. (2000) showed that the ability of *R*-(+)-WIN-55212 to suppress tremor in CREAE mice could be attenuated by pretreatment (5 mg/kg i.v.) with either the CB_1-selective antagonist/inverse agonist SR141716A or the CB_2-selective antagonist/inverse agonist SR144528. They also found that limb spasticity in CREAE mice could be reduced both by a CB_1-selective agonist (*R*-(+)-methanandamide at 5 mg/kg i.v.) and by a CB_2-selective agonist (JWH-133 at 1.5 mg/kg i.v.). Although CB_2 receptors may be present within the CNS, they are located there and peripherally on immune cells, rather than on neurones (Kearn & Hillard, 1999; Pertwee, 1997), raising the question of how activation of CB_2 receptors could decrease spasticity or tremor in CREAE mice. Also still to be investigated is the part played by "CB_2-like" receptors in the decrease in spasticity or tremor observed by Baker et al. (2000). The existence of such receptors has been proposed by Calignano et al. (1998) to explain the ability of SR144528 to oppose the antinociceptive effect in mice of palmitoylethanolamide, a fatty acid amide that lacks significant affinity for CB_2 (or CB_1) receptors. It has been found by Baker et al. (2000) that at 10 mg/kg i.v., palmitoylethanolamide shares the ability of CB_1 and CB_2 receptor agonists to reduce limb spasticity in CREAE mice.

Baker et al. (2000) have also found that both SR141716A and SR144528 can increase spasticity in CREAE mice. More specifically, when administered by itself at 5 mg/kg i.v., SR141716A was found to exacerbate markedly limb spasticity in mildly spastic CREAE mice. At the same dose, SR144528 produced increases in hind-limb and tail spasticity, and enhanced increases in limb spasticity induced by SR141716A. By itself, SR141716A also provoked forelimb tremor in some tremor-free CREAE mice. These effects of SR141716A and SR144528 could have stemmed, at least in part, from the presence of CB_1 and CB_2 receptors that are spontaneously coupled to their effector mechanisms (constitutively active receptors). Thus, there is evidence that rather than being "silent" antagonists, SR141716A and SR144528 are "inverse agonists" with the ability to reduce the constitutive activity of cannabinoid receptors (Bouaboula et al., 1997; Coutts et al., 2000; MacLennan et al., 1998; Pan et al., 1998; Portier et al., 1999; Rinaldi-Carmona et al., 1998; Sim-Selley et al., 2001). It is also possible that spasticity and tremor in CREAE mice is attenuated to some extent by endocannabinoids released onto cannabinoid receptors, and that SR141716A and SR144528 reduce this attenuation by competing for these receptors. This hypothesis is supported by three observations (Baker et al., 2001), the first of which is that spastic CREAE mice have elevated concentrations of the endocannabinoids, anandamide and 2-arachidonoylglycerol, in their brains and spinal cords. Palmitoylethanolamide levels are also elevated in the spinal cords of spastic CREAE mice, although not in the brains of these animals. The second of these observations is that spasticity in CREAE mice can be ameliorated by drugs expected to augment extracellular concentrations of endocannabinoids. These drugs are *N*-(4-hydroxyphenyl) arachidonylamide, an inhibitor of endocannabinoid membrane transport, and palmitylsulphonyl fluoride, which inhibits the enzymic hydrolysis of endocannabinoids (see

also Pertwee, 2000). The third observation is that limb and tail spasticity in CREAE mice can be transiently increased by rolipram, a selective inhibitor of cyclic AMP-selective phosphodiesterase IV that is expected to disrupt cannabinoid receptor signalling by counteracting cannabinoid receptor-mediated inhibition of cyclic AMP production (both CB_1 and CB_2 receptors are negatively coupled to adenylate cyclase) (Felder et al., 1995; Howlett & Fleming, 1984; Slipetz et al., 1995; see also Pertwee, 1997). When taken together, the findings of Baker et al. (2000, 2001) provide convincing evidence for the tonic control of spasticity by the endocannabinoid system, at least in CREAE mice. Future research should establish whether or not a similar mechanism operates in multiple sclerosis. It should also more precisely identify the locations within the brain and spinal cord at which endocannabinoid concentrations increase in CREAE mice, and determine the extent to which these locations are restricted to sites within the brain and spinal cord responsible for the regulation of motor function. In addition, it will be of interest to discover the mechanisms responsible for this increase in endocannabinoid production and to determine what effect, if any, the increase has on cannabinoid receptor density or signalling in CREAE mice. Interestingly, there is already evidence for a decrease in the concentrations of CB_1 receptor mRNA and cannabinoid-binding sites in striatal and cortical regions of the brains of EAE rats and for an increase in the coupling efficiency of the cannabinoid receptors still present in these brain areas (Berrendero et al., 2001). Whether these receptor changes are causally linked to any increases in endocannabinoid concentrations that may have occurred in the brains of these animals has yet to be established. The physiological consequences of these opposing changes in cannabinoid receptor density and signalling on brain function has also still to be investigated.

The clinical evidence that cannabinoids can relieve muscle spasms and certain other signs of motor dysfunction caused by multiple sclerosis or spinal cord injury is also supported by other observations from animal experiments. Thus, there is well-established evidence that cannabinoid receptor agonists suppress the perception of painful stimuli by animals in models of both acute pain and inflammatory and neuropathic pain (see Pertwee, 2001). There are also reports that cannabinoids can suppress spinal reflexes in cats (Tramposch et al., 1981; Turkanis & Karler, 1983, 1986; see also Pertwee, 1988) and can produce marked catalepsy and/or hypokinesia in dogs, rats, and mice (Martin et al., 1995; Razdan, 1986). In addition, Richter and Löscher (1994) have found that the synthetic cannabinoid receptor agonist *R*-(+)-WIN-55212 can decrease the severity of dystonia in mutant Syrian hamsters with primary generalized dystonia. Interestingly, the hamster experiments also yielded data indicating that when sub-effective doses of *R*-(+)-WIN-55212 and diazepam are co-administered, they can interact synergistically to produce significant antidystonic effects. This finding is in line with other reports that cannabinoids interact synergistically with both benzodiazepines and γ-aminobutyric acid receptor agonists to alter motor function in rats and mice (Pertwee & Greentree, 1988; Pertwee et al., 1988; Pertwee & Wickens, 1991; see also Pertwee, 1992). Cannabinoid-induced catalepsy and hypokinesia are most likely mediated by CB_1 receptors that are found in high concentrations in many of the brain areas that regulate motor function, particularly in the substantia nigra pars reticulata, entopeduncular nucleus, globus pallidus, lateral caudate-putamen, and the molecular layer of the cerebellum (Herkenham et al., 1991; see also Pertwee, 1997). Whether these brain areas are also where cannabinoids act to produce their putative spasticity-reducing effect remains to be established. Other possibilities that cannabinoids can reduce spasticity by acting on the terminals of motoneurones (Van der Kloot, 1994) or by interacting with spinal pathways also require further investigation. The antinociceptive effects of cannabinoids are also mediated by cannabinoid receptors, in this case, by CB_1 receptors located on pain pathways in the brain and spinal cord and on the peripheral terminals of primary sensory neurones and possibly also by CB_2 or CB_2-like receptors (see Pertwee, 2001).

In view of anecdotal claims and evidence from controlled clinical studies that cannabis, Δ^9-THC, and nabilone can improve bladder function in some patients with multiple sclerosis or spinal cord injury (Sections 2 and 3), it is also noteworthy that cannabinoid receptor agonists inhibit electrically evoked contractions of mouse isolated urinary bladder (Martin et al., 2000; Pertwee, 1997; Pertwee & Fernando, 1996). This they seem to do by acting on prejunctional neuronal CB_1 receptors to inhibit the evoked release of contractile transmitters. The cannabinoid receptor agonist *R*-(+)-WIN-55212 has also been found to inhibit neuronally evoked contractions of urinary bladder sections of rat, although not of dog, pig, cynomolgus monkey, or human (Martin et al., 2000). Further experiments are required to establish the basis of this species difference.

Finally, Molina-Holgado et al. (1998) have performed experiments with primary astrocyte cultures prepared from 1-day-old postnatal mouse cerebral cortex infected with Theiler's murine encephalomyelitis virus (TMEV), a treatment that induces multiple sclerosis-like demyelination and enhances astrocyte production of interleukin-6. They found that the endogenous cannabinoid receptor agonist anandamide enhanced the release of interleukin-6 from the TMEV-infected astrocytes, and that this effect could be blocked by the selective CB_1 receptor antagonist/inverse agonist SR141716A at 1 μM. The role of interleukin-6 in multiple sclerosis remains to be established. However, there is evidence that this cytokine has neuroprotective properties and that it may promote neural repair (see Molina-Holgado et al., 1998). It has also been reported that administration of human recombinant interleukin-6 reduces demyelination and inflammation in the spinal cord of TMEV-infected mice (Rodriguez et al., 1994).

5. Conclusions

Although the evidence that cannabis and individual cannabinoids are effective against the muscle spasticity/spasm and pain of multiple sclerosis and spinal cord injury is not conclusive, it is sufficient to warrant clinical trials with cannabinoids that will provide more substantial clinical data, both about the efficacy of cannabinoids and about their unwanted effects. The case for such trials is reinforced by the need for treatments that are more effective and that produce less unpleasant side effects than those now used to manage symptoms of multiple sclerosis and spinal cord injury. Particularly important steps in the design of clinical trials will be the selection of the drug(s) to be investigated, the mode of administration of this drug(s), and the dose levels to be used. Apart from the lack of good modes of delivery for cannabinoids (see Section 3), practical difficulties confronting the design of clinical trials include the dearth of sensitive and reliable objective measures of spasticity and rigidity and the problem of devising an adequate placebo control for drugs that produce such marked and characteristic psychotropic effects. In addition, cannabinoid elimination from the body is rather slow (Agurell et al., 1986), necessitating lengthy wash-out periods between treatments if a cross-over design is used. In spite of these difficulties, it is encouraging that the British Medical Research Council recently funded a 3-year multicentre clinical trial with 660 patients that is to be directed at investigating the abilities of oral cannabis and Δ^9-THC to relieve signs and symptoms of multiple sclerosis (Dyer, 2001). Clinical studies with multiple sclerosis patients are also being conducted in the United Kingdom using novel delivery systems to administer cannabis extracts (Whittle et al., 2001; see also Section 3).

Another important area for future clinical research must be the development of strategies that maximize separation between the sought-after therapeutic effects of cannabinoids and the unwanted effects of these drugs, particularly their psychotropic effects. One strategy may be to use drugs that activate the endogenous cannabinoid system indirectly by increasing extracellular levels of endocannabinoids through inhibition of their membrane transport or enzymic hydrolysis, and, indeed, drugs of this kind are already available (Pertwee, 2000). It will be important to establish whether, as in CREAE mice (Baker et al., 2001), endocannabinoid production increases during periods of spasticity in humans. If such increases do occur in multiple sclerosis, ideally they should be located predominantly at sites at which spasticity is suppressed, as this should render inhibitors of endocannabinoid membrane transport or enzymic hydrolysis significantly more selective than direct cannabinoid receptor agonists. Another possibility is to administer a cannabinoid in combination with a second agent that augments only the sought-after effects of the cannabinoid. Thus, there already is evidence from animal experiments that synergistic interactions can occur between cannabinoids and opioids for analgesia (Pertwee, 2001; Welch & Stevens, 1992) and between cannabinoids and benzodiazepines for depressant effects on motor function (Pertwee, 1992; Richter & Löscher, 1994). As there are claims by multiple sclerosis patients that cannabis can relieve their symptoms at dose levels that do not induce a 'high,' a third strategy may be to administer an agonist (partial agonist) with a reduced ability (efficacy) to activate CB_1 receptors. This approach assumes that it should be possible to develop a partial agonist that has sufficient efficacy to relieve muscle spasticity/spasm and pain, but insufficient efficacy to produce a full range of cannabimimetic psychotropic effects, even when it occupies all available CB_1 receptors. Possible lead compounds are 6′-azidohex-2′-yne-Δ^8-THC and 6′-azidohex-*cis*-2′-ene-Δ^8-THC (Ross et al., 1999). Since there is evidence that CB_2 or CB_2-like receptors can mediate suppression of limb spasticity and/or tremor, at least in CREAE mice (Baker et al., 2000), it will also be worth carrying out clinical studies with CB_2-selective agonists. It should be noted, however, that although such agonists are expected to lack psychotropic properties, current knowledge about the pharmacology and toxicology of CB_2 receptor agonists is far from complete. Because CB_2 receptors are located mainly in the immune system (Galiègue et al., 1995; Munro et al., 1993; see also Pertwee, 1997) and multiple sclerosis is considered to be an immune disorder, the possibility also exists that CB_2 receptor agonists (or antagonists) could be used to slow, or even halt, the course of this disease.

References

Achiron, A., Miron, S., Lavie, V., Margalit, R., & Biegon, A. (2000). Dexanabinol (HU-211) effect on experimental autoimmune encephalomyelitis: implications for the treatment of acute relapses of multiple sclerosis. *J Neuroimmunol 102*, 26–31.

Agurell, S., Halldin, M., Lindgren, J.-E., Ohlsson, A., Widman, M., Gillespie, H., & Hollister, L. (1986). Pharmacokinetics and metabolism of Δ^1-tetrahydrocannabinol and other cannabinoids with emphasis on man. *Pharmacol Rev 38*, 21–43.

Baker, D., Pryce, G., Croxford, J. L., Brown, P., Pertwee, R. G., Huffman, J. W., & Layward, L. (2000). Cannabinoids control spasticity and tremor in a multiple sclerosis model. *Nature 404*, 84–87.

Baker, D., Pryce, G., Croxford, J. L., Brown, P., Pertwee, R. G., Makriyannis, A., Khanolkar, A., Layward, L., Fezza, F., Bisogno, T., & Di Marzo, V. (2001). Endocannabinoids control spasticity in a multiple sclerosis model. *FASEB J 15*, 300–302.

Berrendero, F., Sánchez, A., Cabranes, A., Puerta, C., Ramos, J. A., Gárcia-Merino, A., & Fernández-Ruiz, J. (2001). Changes in cannabinoid CB_1 receptors in striatal and cortical regions of rats with experimental allergic encephalomyelitis, an animal model of multiple sclerosis. *Synapse 41*, 195–202.

Bouaboula, M., Perrachon, S., Milligan, L., Canat, X., Rinaldi-Carmona, M., Portier, M., Barth, F., Calandra, B., Pecceu, F., Lupker, J., Maffrand, J.-P., Le Fur, G., & Casellas, P. (1997). A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. *J Biol Chem 272*, 22330–22339.

Brenneisen, R., Egli, A., ElSohly, M. A., Henn, V., & Spiess, Y. (1996). The effect of orally and rectally administered Δ^9-tetrahydrocannabinol

on spasticity: a pilot study with 2 patients. *Int J Clin Pharmacol Ther 34*, 446–452.

Calignano, A., La Rana, G., Giuffrida, A., & Piomelli, D. (1998). Control of pain initiation by endogenous cannabinoids. *Nature 394*, 277–281.

Clifford, D. B. (1983). Tetrahydrocannabinol for tremor in multiple sclerosis. *Ann Neurol 13*, 669–671.

Consroe, P., Musty, R., Rein, J., Tillery, W., & Pertwee, R. (1997). The perceived effects of smoked cannabis on patients with multiple sclerosis. *Eur Neurol 38*, 44–48.

Coutts, A. A., Brewster, N., Ingram, T., Razdan, R. K., & Pertwee, R. G. (2000). Comparison of novel cannabinoid partial agonists and SR141716A in the guinea-pig small intestine. *Br J Pharmacol 129*, 645–652.

Dunn, M., & Davis, R. (1974). The perceived effects of marijuana on spinal cord injured males. *Paraplegia 12*, 175.

Dyer, O. (2001). Cannabis trial launched in patients with MS. *Br Med J 322*, 192.

Felder, C. C., Joyce, K. E., Briley, E. M., Mansouri, J., Mackie, K., Blond, O., Lai, Y., Ma, A. L., & Mitchell, R. L. (1995). Comparison of the pharmacology and signal transduction of the human cannabinoid CB_1 and CB_2 receptors. *Mol Pharmacol 48*, 443–450.

Fung, M., Gallagher, C., & Machtay, M. (1999). Lung and aero-digestive cancers in young marijuana smokers. *Tumori 85*, 140–142.

Galiègue, S., Mary, S., Marchand, J., Dussossoy, D., Carrière, D., Carayon, P., Bouaboula, M., Shire, D., Le Fur, G., & Casellas, P. (1995). Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Biochem 232*, 54–61.

Greenberg, H. S., Werness, S. A. S., Pugh, J. E., Andrus, R. O., Anderson, D. J., & Domino, E. F. (1994). Short-term effects of smoking marijuana on balance in patients with multiple sclerosis and normal volunteers. *Clin Pharmacol Ther 55*, 324–328.

Hamann, W., & di Vadi, P. P. (1999). Analgesic effect of the cannabinoid analogue nabilone is not mediated by opioid receptors. *Lancet 353*, 560.

Herkenham, M., Lynn, A. B., Johnson, M. R., Melvin, L. S., de Costa, B. R., & Rice, K. C. (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J Neurosci 11*, 563–583.

Hodges, C. (1993, February 23). I wish I could get it at the chemist's. *The Independent*.

Holdcroft, A., Smith, M., Jacklin, A., Hodgson, H., Smith, B., Newton, M., & Evans, F. (1997). Pain relief with oral cannabinoids in familial Mediterranean fever. *Anaesthesia 52*, 483–488.

Hollister, L. E. (1986). Health aspects of cannabis. *Pharmacol Rev 38*, 1–20.

Howlett, A. C., & Fleming, R. M. (1984). Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. *Mol Pharmacol 26*, 532–538.

Jain, A. K., Ryan, J. R., McMahon, F. G., & Smith, G. (1981). Evaluation of intramuscular levonantradol and placebo in acute postoperative pain. *J Clin Pharmacol 21* (suppl. 8 & 9), 320S–326S.

Kearn, C. S., & Hillard, C. J. (1999). A model for the study of cannabinoid actions in microglia. In *Abstracts of the 1999 Symposium on the Cannabinoids* (p. 44). Burlington: International Cannabinoid Research Society.

Lyman, W. D., Sonett, J. R., Brosnan, C. F., Elkin, R., & Bornstein, M. B. (1989). Δ^9-Tetrahydrocannabinol: a novel treatment for experimental autoimmune encephalomyelitis. *J Neuroimmunol 23*, 73–81.

MacLennan, S. J., Reynen, P. H., Kwan, J., & Bonhaus, D. W. (1998). Evidence for inverse agonism of SR141716A at human recombinant cannabinoid CB_1 and CB_2 receptors. *Br J Pharmacol 124*, 619–622.

Malec, J., Harvey, R. F., & Cayner, J. J. (1982). Cannabis effect on spasticity in spinal cord injury. *Arch Phys Med Rehabil 63*, 116–118.

Martin, B. R., Thomas, B. F., & Razdan, R. K. (1995). Structural requirements for cannabinoid receptor probes. In R. G. Pertwee (Ed.), *Cannabinoid Receptors* (pp. 35–85). London: Academic Press.

Martin, R. S., Luong, L. A., Welsh, N. J., Eglen, R. M., Martin, G. R., & MacLennan, S. J. (2000). Effects of cannabinoid receptor agonists on neuronally-evoked contractions of urinary bladder tissues isolated from rat, mouse, pig, dog, monkey and human. *Br J Pharmacol 129*, 1707–1715.

Martyn, C. N., Illis, L. S., & Thom, J. (1995). Nabilone in the treatment of multiple sclerosis. *Lancet 345*, 579.

Maurer, M., Henn, V., Dittrich, A., & Hofmann, A. (1990). Delta-9-tetrahydrocannabinol shows antispastic and analgesic effects in a single case double-blind trial. *Eur Arch Psychiatry Clin Neurosci 240*, 1–4.

Mechoulam, R. (1986). The pharmacohistory of *Cannabis sativa*. In R. Mechoulam (Ed.), *Cannabinoids as Therapeutic Agents* (pp. 1–19). Boca Raton: CRC Press.

Meinck, H.-M., Schönle, P. W., & Conrad, B. (1989). Effect of cannabinoids on spasticity and ataxia in multiple sclerosis. *J Neurol 236*, 120–122.

Molina-Holgado, F., Molina-Holgado, E., & Guaza, C. (1998). The endogenous cannabinoid anandamide potentiates interleukin-6 production by astrocytes infected with Theiler's murine encephalomyelitis virus by a receptor-mediated pathway. *FEBS Lett 433*, 139–142.

Munro, S., Thomas, K. L., & Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature 365*, 61–65.

Notcutt, W., Price, M., Blossfeldt, P., & Chapman, G. (1999). Clinical experience of the synthetic cannabinoid nabilone for chronic pain. In G. G. Nahas, K. M. Sutin, D. Harvey, & S. Agurell (Eds.), *Marihuana and Medicine* (pp. 567–572). Totowa: Humana Press (Ch. 47).

Noyes, R., Brunk, S. F., Avery, D. H., & Canter, A. (1975a). The analgesic properties of delta-9-tetrahydrocannabinol and codeine. *Clin Pharmacol Ther 18*, 84–89.

Noyes, R., Brunk, S. F., Baram, D. A., & Canter, A. (1975b). Analgesic effect of delta-9-tetrahydrocannabinol. *J Clin Pharmacol 15*, 139–143.

Pan, X., Ikeda, S. R., & Lewis, D. L. (1998). SR 141716A acts as an inverse agonist to increase neuronal voltage-dependent Ca^{2+} currents by reversal of tonic CB1 cannabinoid receptor activity. *Mol Pharmacol 54*, 1064–1072.

Paton, W. D. M., & Pertwee, R. G. (1973). The actions of cannabis in man. In R. Mechoulam (Ed.), *Marijuana* (pp. 287–333). New York: Academic Press.

Pertwee, R. G. (1988). The central neuropharmacology of psychotropic cannabinoids. *Pharmacol Ther 36*, 189–261.

Pertwee, R. G. (1992). *In vivo* interactions between psychotropic cannabinoids and other drugs involving central and peripheral neurochemical mediators. In L. Murphy, & A. Bartke (Eds.), *Marijuana/Cannabinoids. Neurobiology and Neurophysiology* (pp. 165–218). Boca Raton: CRC Press.

Pertwee, R. G. (1997). Pharmacology of cannabinoid CB_1 and CB_2 receptors. *Pharmacol Ther 74*, 129–180.

Pertwee, R. G. (2000). Cannabinoid receptor ligands: clinical and neuropharmacological considerations, relevant to future drug discovery and development. *Expert Opin Invest Drugs 9*, 1553–1571.

Pertwee, R. G. (2001). Cannabinoid receptors and pain. *Prog Neurobiol 63*, 569–611.

Pertwee, R. G., & Fernando, S. R. (1996). Evidence for the presence of cannabinoid CB_1 receptors in mouse urinary bladder. *Br J Pharmacol 118*, 2053–2058.

Pertwee, R. G., & Greentree, S. G. (1988). Delta-9-tetrahydrocannabinol-induced catalepsy in mice is enhanced by pretreatment with flurazepam or chlordiazepoxide. *Neuropharmacology 27*, 485–491.

Pertwee, R. G., & Wickens, A. P. (1991). Enhancement by chlordiazepoxide of catalepsy induced in rats by intravenous or intrapallidal injections of enantiomeric cannabinoids. *Neuropharmacology 30*, 237–244.

Pertwee, R. G., Greentree, S. G., & Swift, P. A. (1988). Drugs which stimulate or facilitate central GABAergic transmission interact synergistically with delta-9-tetrahydrocannabinol to produce marked catalepsy in mice. *Neuropharmacology 27*, 1265–1270.

Pertwee, R. G., Gibson, T. M., Stevenson, L. A., Ross, R. A., Banner, W. K., Saha, B., Razdan, R. K., & Martin, B. R. (2000). O-1057, a potent water-soluble cannabinoid receptor agonist with antinociceptive properties. *Br J Pharmacol 129*, 1577–1584.

Petro, D. J., & Ellenberger, C. (1981). Treatment of human spasticity with Δ^9-tetrahydrocannabinol. *J Clin Pharmacol 21* (suppl. 8 & 9), 413S–416S.

Portier, M., Rinaldi-Carmona, M., Pecceu, F., Combes, T., Poinot-Chazel, C., Calandra, B., Barth, F., Le Fur, G., & Casellas, P. (1999). SR 144528, an antagonist for the peripheral cannabinoid receptor that behaves as an inverse agonist. *J Pharmacol Exp Ther 288*, 582–589.

Razdan, R. K. (1986). Structure-activity relationships in cannabinoids. *Pharmacol Rev 38*, 75–149.

Richter, A., & Lõscher, W. (1994). (+)-WIN 55,212-2, a novel cannabinoid receptor agonist, exerts antidystonic effects in mutant dystonic hamsters. *Eur J Pharmacol 264*, 371–377.

Rinaldi-Carmona, M., Barth, F., Millan, J., Derocq, J.-M., Casellas, P., Congy, C., Oustric, D., Sarran, M., Bouaboula, M., Calandra, B., Portier, M., Shire, D., Brelière, J.-C., & Le Fur, G. (1998). SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J Pharmacol Exp Ther 284*, 644–650.

Rodriguez, M., Pavelko, K. D., McKinney, C. W., & Leibowitz, J. L. (1994). Recombinant human IL-6 suppresses demyelination in a viral model of multiple sclerosis. *J Immunol 153*, 3811–3821.

Ross, R. A., Gibson, T. M., Stevenson, L. A., Saha, B., Crocker, P., Razdan, R. K., & Pertwee, R. G. (1999). Structural determinants of the partial agonist-inverse agonist properties of 6′-azidohex-2′-yne-Δ^8-tetrahydrocannabinol at cannabinoid receptors. *Br J Pharmacol 128*, 735–743.

Schnelle, M., Grotenhermen, F., Reif, M., & Gorter, R. W. (1999). Results of a standardized survey on the medical use of cannabis products in the German-speaking area. *Forsch Komplementärmed 6* (suppl. 3), 28–36.

Schon, F., Hart, P. E., Hodgson, T. L., Pambakian, A. L. M., Ruprah, M., Williamson, E. M., & Kennard, C. (1999). Suppression of pendular nystagmus by smoking cannabis in a patient with multiple sclerosis. *Neurology 53*, 2209–2210.

Sherman, M. P., Campbell, L. A., Gong, H., Roth, M. D., & Tashkin, D. P. (1991). Antimicrobial and respiratory burst characteristics of pulmonary alveolar macrophages recovered from smokers of marijuana alone, smokers of tobacco alone, smokers of marijuana and tobacco, and nonsmokers. *Am Rev Respir Dis 144*, 1351–1356.

Sim-Selley, L. J., Brunk, L. K., & Selley, D. E. (2001). Inhibitory effects of SR141716A on G-protein activation in rat brain. *Eur J Pharmacol 414*, 135–143.

Slipetz, D. M., O'Neill, G. P., Favreau, L., Dufresne, C., Gallant, M., Gareau, Y., Guay, D., Labelle, M., & Metters, K. M. (1995). Activation of the human peripheral cannabinoid receptor results in inhibition of adenylyl cyclase. *Mol Pharmacol 48*, 352–361.

Tramposch, A., Sangdee, C., Franz, D. N., Karler, R., & Turkanis, S. A. (1981). Cannabinoid-induced enhancement and depression of cat monosynaptic reflexes. *Neuropharmacology 20*, 617–621.

Turkanis, S. A., & Karler, R. (1983). Effects of Δ^9-tetrahydrocannabinol on cat spinal motoneurons. *Brain Res 288*, 283–287.

Turkanis, S. A., & Karler, R. (1986). Cannabidiol-caused depression of spinal motoneuron responses in cats. *Pharmacol Biochem Behav 25*, 89–94.

Ungerleider, J. T., Andyrsiak, T., Fairbanks, L., Ellison, G. W., & Myers, L. W. (1987). Delta-9-THC in the treatment of spasticity associated with multiple sclerosis. *Adv Alcohol Subst Abuse 7*, 39–50.

Van der Kloot, W. (1994). Anandamide, a naturally-occurring agonist of the cannabinoid receptor, blocks adenylate cyclase at the frog neuromuscular junction. *Brain Res 649*, 181–184.

Welch, S. P., & Stevens, D. L. (1992). Antinociceptive activity of intrathecally administered cannabinoids alone, and in combination with morphine, in mice. *J Pharmacol Exp Ther 262*, 10–18.

Whittle, B. A., Guy, G. W., & Robson, P. (2001). Prospects for new cannabis-based prescription medicines. *J Cannabis Ther 1*, 183–205.

Wirguin, I., Mechoulam, R., Breuer, A., Schezen, E., Weidenfeld, J., & Brenner, T. (1994). Suppression of experimental autoimmune encephalomyelitis by cannabinoids. *Immunopharmacology 28*, 209–214.

CURRENT LITERATURE

CANNABINOIDS: DEFENDING THE EPILEPTIC BRAIN

The Endogenous Cannabinoid System Regulates Seizure Frequency and Duration in a Model of Temporal Lobe Epilepsy

Wallace MJ, Blair RE, Falenski KW, Martin BR, DeLorenzo RJ

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Several lines of evidence suggest that cannabinoid compounds are anticonvulsant. However, the anticonvulsant potential of cannabinoids and, moreover, the role of the endogenous cannabinoid system in regulating seizure activity have not been tested in an in vivo model of epilepsy that is characterized by spontaneous, recurrent seizures. Here, by using the rat pilocarpine model of epilepsy, we show that the marijuana extract 9-tetrahydrocannabinol (10 mg/kg) as well as the cannabimimetic, 4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6*H*-pyrrolo[3,2,1-*i,j*]quinolin-6-one [*R*(+)WIN55,212 (5 mg/kg)], completely abolished spontaneous epileptic seizures. Conversely, application of the cannabinoid CB1 receptor (CB1) antagonist, *N*-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamidehydrochloride (SR141716A), significantly increased both seizure duration and frequency. In some animals, CB1-receptor antagonism resulted in seizure durations that were protracted to a level consistent with the clinical condition status epilepticus. Furthermore, we determined that during an short-term pilocarpine-induced seizure, levels of the endogenous CB1 ligand 2-arachidonylglycerol increased significantly within the hippocampal brain region. These data not only indicate anticonvulsant activity of exogenously applied cannabinoids but also suggest that endogenous cannabinoid tone modulates seizure termination and duration through activation of the CB1 receptor. Western blot and immunohistochemical analyses revealed that CB1-receptor protein expression was significantly increased throughout the CA regions of epileptic hippocampi. By demonstrating a role for the endogenous cannabinoid system in regulating seizure activity, these studies define a role for the endogenous cannabinoid system in modulating neuroexcitation and suggest that plasticity of the CB1-receptor occurs with epilepsy.

CB1 Cannabinoid Receptors and On-demand Defense Against Excitotoxicity

Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, Azad SC, Cascio MG, Gutierrez SO, van der Stelt M, Lopez-Rodriguez ML, Casanova E, Schutz G, Zieglgansberger W, Di Marzo V, Behl C, Lutz B

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Abnormally high spiking activity can damage neurons. Signaling systems to protect neurons from the consequences of abnormal discharge activity have been postulated. We generated conditional mutant mice that lack expression of the cannabinoid receptor type 1 in principal forebrain neurons but not in adjacent inhibitory interneurons. In mutant mice, the excitotoxin kainic acid (KA) induced excessive seizures in vivo. The threshold to KA-induced neuronal excitation in vitro was severely reduced in hippocampal pyramidal neurons of mutants. KA administration rapidly increased hippocampal levels of anandamide and induced protective mechanisms in wild-type principal hippocampal neurons. These protective mechanisms could not be triggered in mutant mice. The endogenous cannabinoid system thus provides on-demand protection against acute excitotoxicity in central nervous system neurons.

COMMENTARY

It has been known for centuries that exogenous cannabinoids, such as tetrahydrocannabinol (the major constituent of cannabis), have anticonvulsant activity, but little is known about the molecular mechanisms of the cannabinoid system. In mammals, a number of endogenous cannabinoids have been identified, including 2-arachidonoylglycerol (2-AG) and anandamide. Endocannabinoids, like neurotransmitters, are released from neurons after membrane depolarization and Ca^{2+}

influx (1). The cannabinoids work through CB1 receptors centrally and CB2 receptors peripherally. The CB1 receptor is the most highly expressed G protein–coupled receptor in the brain and has been implicated in regulation of neuronal excitability (2). Two recent studies have advanced our understanding of the endogenous cannabinoid system and renewed the interest in cannabinoids as a potential treatment for epilepsy. Both studies show that the endogenous cannabinoid system is rapidly activated after seizure activity. Wallace and colleagues demonstrated that exogenous cannabinoids effectively control seizures in a rat epilepsy model. Marsicano and colleagues extended the use of experimental animal models to determine which neuronal circuits are involved in the anticonvulsant effect of cannabinoids. The investigators used conditional knockout mice to show that the glutamatergic neurons of the forebrain are principally responsible for cannabinoid-mediated protection against seizures.

Wallace et al. used a rat model of temporal lobe epilepsy in which animals have seizures for life after treatment with pilocarpine. Unlike standard antiepileptic drugs [AEDs; e.g., phenobarbital (PB) and phenytoin (PHT)], cannabinoids were very effective AEDs in this rat model. This finding implies that cannabinoids may offer unique advantages in treating seizures refractory to currently prescribed AEDs. Blocking the CB1 receptor increased both seizure frequency and duration in epileptic rats but did not cause seizures in control rats, suggesting that CB1 activation is a response to seizure activity rather than a cause. In support of this idea, the authors found that a single pilocarpine-induced seizure increased levels of 2-AG in the hippocampus within 15 minutes.

Marsicano et al. also measured levels of endogenous cannabinoids in the hippocampus of mice after kainic acid–induced seizures. Unlike in the pilocarpine rat model, they saw no increase in 2-AG, but another cannabinoid, anandamide, showed transiently increased levels, which peaked 20 minutes after injection. Different processing of endocannabinoids in different species and/or different experimental conditions (e.g., kainic acid vs. pilocarpine) may be responsible for these differences. However, both studies showed rapid endocannabinoid increase in response to seizures. This finding further supports the involvement of endogenous cannabinoids in protection against seizure activity. The mechanisms causing this increase in endocannabinoid levels in the brain are still to be determined, but it could be due to enhanced production, decreased degradation, or enhanced synthesis of cannabinoid precursors.

In addition to elevated endocannabinoid levels, Wallace et al. reported that seizure activity increased levels of CB1-receptor protein in the CA1 through CA3 regions of the hippocampus. This supports the idea that plasticity of the endogenous cannabinoid system occurs in the hippocampus in response to seizures. Animals were studied for up to 1 year, suggesting the increase in CB1-receptor expression is prolonged and probably permanent. To determine which neural circuits were involved in CB1-receptor activation in response to seizures, Marsicano et al. produced a conditional knockout of the CB1 receptor, in which CB1 was deleted in principal glutamatergic neurons of the forebrain but still expressed in cortical γ-aminobutyric acid (GABA)ergic interneurons. Complete knockout of the CB1 receptor resulted in increased seizure severity. This finding also was observed in the conditional knockouts, implying that the principal neurons of the forebrain are necessary for neuroprotection.

How does CB1-receptor activation provide neuroprotection? In vitro, exogenously applied cannabinoids decrease neuronal excitability and inhibit glutamatergic transmission (3). Marsicano et al. measured glutamatergic excitation of CA1 pyramidal neurons in an in vitro hippocampal slice preparation. Kainic acid significantly increased the glutamatergic excitation of neurons obtained from conditional CB1 knockout mice, compared with controls. Therefore endogenously released cannabinoids might provide neuroprotection by CB1 receptor–mediated inhibition of glutamatergic transmission. Marsicano and colleagues also found evidence that CB1 receptors activate intracellular signaling cascades, which may contribute to long-term adaptive cellular changes in response to the seizure.

These studies show that seizures rapidly activate the endogenous cannabinoid system, which provides protection against excessive neuronal activity by reducing excitability of hippocampal pyramidal neurons and activating intracellular signaling cascades. Furthermore, CB1 receptors on principal glutamatergic neurons of the forebrain are primarily responsible for this action. These studies have improved our knowledge of the endocannabinoid system, but further investigation is required. What causes endocannabinoid levels to increase in response to seizures? Which endocannabinoid is important in humans: 2-AG, anandamide, or another CB1-receptor ligand? Can we target therapy to the critical circuits in the forebrain? Smoking marijuana is obviously is not an appropriate therapy; in addition to the psychoactive effects, the inhalation of smoke poses obvious health risks. To this end, drug companies have already isolated the active ingredients in cannabis and produced them in the form of a pill or a spray. However, most synthetic cannabinoids still have psychoactive effects and are undesirable for therapeutics. It may be more beneficial to target cannabinoid transport or degradation systems to increase the levels of endogenous cannabinoids. Enhancing the cannabinoid system may prove to be an effective treatment for epilepsy, especially in cases in which standard drugs fail to control seizures.

by Robyn Wallace, Ph.D.

References

1. Di Marzo V, Melck D, Bisogno T, De Petrocellis L. Endocannabinoids: Endogenous cannabinoid receptor ligands with neuromodulatory action. Trends Neurosci 1998;21:521–528.
2. Wilson RI, Nicoll RA. Endogenous cannabinoids mediate retrograde signaling at hippocampal synapses. Nature 2001;410:588–592.
3. van der Stelt M, Veldhuis WB, Maccarrone M, Bar PR, Nicolay K, Veldink GA, Di Marzo V, Vliegenthart JF. Acute neuronal injury, excitotoxicity, and the endocannabinoid system. Mol Neurobiol 2002;26:317–346.

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The Endogenous Cannabinoid System Regulates Seizure Frequency and Duration in a Model of Temporal Lobe Epilepsy

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ABSTRACT

Several lines of evidence suggest that cannabinoid compounds are anticonvulsant. However, the anticonvulsant potential of cannabinoids and, moreover, the role of the endogenous cannabinoid system in regulating seizure activity has not been tested in an in vivo model of epilepsy that is characterized by spontaneous, recurrent seizures. Here, using the rat pilocarpine model of epilepsy, we show that the marijuana extract Δ^9- tetrahydrocannabinol (10 mg/kg) as well as the cannabimimetic, 4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6*H*-pyrrolo[3,2,1-*i,j*]quinolin-6-one [*R*(+)WIN55,212 (5 mg/kg)], completely abolished spontaneous epileptic seizures. Conversely, application of the cannabinoid CB_1 receptor (CB_1) antagonist, *N*-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamidehydrochloride (SR141716A), significantly increased both seizure duration and frequency. In some animals, CB_1 receptor antagonism resulted in seizure durations that were protracted to a level consistent with the clinical condition status epilepticus. Furthermore, we determined that during an short-term pilocarpine-induced seizure, levels of the endogenous CB_1 ligand 2-arachidonylglycerol increased significantly within the hippocampal brain region. These data indicate not only anticonvulsant activity of exogenously applied cannabinoids but also suggest that endogenous cannabinoid tone modulates seizure termination and duration through activation of the CB_1 receptor. Furthermore, Western blot and immunohistochemical analyses revealed that CB_1 receptor protein expression was significantly increased throughout the CA regions of epileptic hippocampi. By demonstrating a role for the endogenous cannabinoid system in regulating seizure activity, these studies define a role for the endogenous cannabinoid system in modulating neuroexcitation and suggest that plasticity of the CB_1 receptor occurs with epilepsy.

Characterized by spontaneously recurrent seizures, epilepsy is one of the most common neurological conditions (Hauser and Hesdorffer, 1990). Understanding the factors that contribute to seizure initiation and termination has important implications for our ability to treat epilepsy and for the potential development of novel anticonvulsant agents. Previous evidence has suggested that the endogenous cannabinoid system may be a novel locus of anticonvulsant activity in the brain (Karler et al., 1974; Wallace et al., 2001). Using the maximal electroshock model of short-term seizure, our laboratory determined that cannabinoid compounds block seizure spread via a cannabinoid CB_1 receptor-dependent mechanism (Wallace et al., 2001, 2002). Further study revealed that application of a CB_1 receptor antagonist lowered the electroshock seizure threshold (Wallace et al., 2002), indicating that elimination of endogenous cannabinoid tone at the CB_1 receptor may increase seizure susceptibility.

The CB_1 receptor is the most highly expressed G-protein-coupled receptor in brain (Herkenham et al., 1990) and has been implicated in regulation of neuronal excitability (Wilson and Nicoll, 2001; Ohno-Shosaku et al., 2002). The endogenous cannabinoids, arachidonylethanolamine and 2-arachidonylglycerol (2-AG) (Devane et al., 1992; Mechoulam et al., 1995), are synthesized "on demand" in response to sustained neuronal depolarization and elevated intracellular calcium levels (Stella et al., 1997); both of these events occur with seizure activity (Hauser and Hesdorffer, 1990; Raza et al., 2001). The neuronal hyperexcitability that accompanies seizure discharge may stimulate endogenous cannabinoid synthesis and subsequently result in CB_1 receptor activation. In light of cannabinoid effects on neurotransmission, increased

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ABBREVIATIONS: 2-AG, 2-arachidonylglycerol; CB_1, cannabinoid CB_1 receptor; EEG, electroencephalographic; *R*(+)WIN55,212, 4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6*H*-pyrrolo[3,2,1-*i,j*]quinolin-6-one; *S*(−)WIN55,212, (*S*)-(−)-[2,3-dihydro-5-methyl-3-([4-morpholinyl]methyl(pyrrolo)-[1,2,3-*d,e*]-(1,4-benzoxazinyl)-[1-napthalenyl]methanone); SR141716A, *N*-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamidehydrochloride; THC, Δ^9-tetrahydrocannabinol; DSI, depolarization-induced suppression of inhibition; SE, status epilepticus; PBS, phosphate-buffered saline; ANOVA, analysis of variance; RM, repeated measures; CA, cornu ammonis.

CB_1 receptor activation could influence seizure activity. However, no studies have evaluated the role of the endogenous cannabinoid system in an intact model of epilepsy.

This study was initiated to evaluate the role of the CB_1 receptor and the endogenous cannabinoid system in regulating seizure activity in a long-term model of epilepsy. We used the pilocarpine model of temporal lobe, partial-complex epilepsy; a rat model of acquired, refractory epilepsy that produces spontaneous recurrent seizures for the lifetime of the animal (Mello et al., 1993; Rice and DeLorenzo, 1998). The pilocarpine model has been shown to closely resemble human refractory partial-complex epilepsy (Mello et al., 1993; Raza et al., 2001). In this study, seizure frequency and duration were determined by continuous electrographic and video recording of each epileptic animal (Rice and DeLorenzo, 1998). The CB_1 receptor agonists *R*(+)WIN55,212 and Δ^9-tetrahydrocannabinol (THC) were evaluated for anticonvulsant efficacy. In addition to agonist effects on seizure activity, the effect of CB_1 receptor antagonism on seizure frequency and duration was evaluated using the specific antagonist SR141716A. Hippocampal levels of 2-AG during short-term, pilocarpine-induced seizures were measured to determine whether a correlation exists between endogenous cannabinoid synthesis and seizure activity. In addition, Western blot and immunohistochemical analyses were used to evaluate hippocampal CB_1 receptor protein expression in the brains of chronically epileptic and sham control rats. The findings presented suggest an anticonvulsant role for the endogenous cannabinoid system and demonstrate that long-term plasticity of the CB_1 receptor occurs with epilepsy.

Materials and Methods

Pilocarpine-Induced Status Epilepticus. Male Sprague-Dawley rats weighing 200 to 250 g were used in accordance with university animal care and use protocols. Animals were housed in single cages on a 12-h/12-h light/dark cycle (lights on at 7:00 AM) and were provided food and water ad libitum. Animals were made epileptic using a modified protocol of Mello et al. (1993) that is well established in our laboratory (Rice and DeLorenzo, 1998). Before pilocarpine injections, animals were administered methylscopolamine nitrate (1 mg/kg i.p.) to minimize peripheral, parasympathetic effects of pilocarpine treatment. Pilocarpine nitrate (375 mg/kg i.p.) was then administered 30 min later. Onset of status epilepticus (SE) typically occurred within 20 to 40 min after pilocarpine injection and was determined when the animal displayed continuous moderate to severe behavioral seizures characterized by forelimb clonus, rearing, and falling.

SE was defined as continuous seizure activity that lasted 30 min or longer or intermittent seizures without regaining consciousness between seizures that lasted 30 min or longer. The severity of convulsions was evaluated, and only those animals that displayed behaviors consistent with ongoing SE were used in the study (Rice and DeLorenzo, 1998). Seizure activity was terminated by consecutive diazepam injections (5 mg/kg i.p., solubilized in 10% ethanol, 45% propylene glycol, and 45% H_2O) at 1, 3, and 5 h post onset of SE. Animals continuing to display seizure activity beyond 6 h post onset of SE were euthanized. Control groups were composed of both naive and sham control animals that received methylscopolamine nitrate and diazepam injections only. Approximately 75% of the SE animals developed epilepsy under these conditions, and the mortality rate from SE was approximately 10%. SE animals that did not stop seizing with diazepam treatment were uncommon and represented less than 2% of the animals injected with pilocarpine.

Epileptic Seizure Monitoring. Seizures were monitored in freely moving animals via simultaneous electroencephalographic (EEG) and video monitoring at least 3 months after pilocarpine treatment (Rice and DeLorenzo, 1998). Electrographic seizures were detected via skull surface electrodes implanted 2 to 3 weeks after the initial episode of SE or after sham treatment in a manner described previously (Perlin et al., 1993). Briefly, animals were put under general ketamine/xylazine anesthesia (75 mg/kg ketamine i.p., 7.5 mg/kg xylazine i.p.), and a midline scalp incision was made to expose the skull. Four surface screw electrodes were implanted bilaterally 2.5 mm from midline, at 2.5 mm posterior to bregma and 2.5 mm anterior to lambda. Surface screw electrodes were connected via Teflon-coated stainless steel wire (Medwire, Mount Vernon, NY) to a male amphenol pin headset assembly, which was secured to the skull with dental acrylic (Hygenic, Akron, OH). Animals were allowed to recover for a minimum of 1 month before experimental analysis. Both electrographic and behavioral seizures were monitored with EEG and video recording, respectively, using a Biomedical Monitoring System Mobile EEG Unit (Campbell, CA).

Seizures were evaluated using established techniques (Rice and DeLorenzo, 1998) and confirmed by an observer blind to experimental treatment. Behavioral epileptic seizures were identified by video analysis of animals displaying moderate to severe behavioral seizures characterized by forelimb clonus, rearing, and falling in conjunction with electrographic seizure activity obtained from EEG analysis.

For single-injection experiments, animals were given a 2 to 3 h equilibration to the treatment setting and then were briefly anesthetized under halothane anesthesia (Halocarbon Laboratories, River Edge, NJ) and injected with either vehicle, *S*(−)WIN55,212 (5 mg/kg i.p.), *R*(+)WIN55,212 (5 mg/kg i.p.), SR141716A (10 mg/kg i.p.), phenobarbital (40 mg/kg i.p.), phenytoin (100 mg/kg i.p.), or THC (30 mg/kg i.p.). All drugs were suspended in a vehicle of absolute ethanol, Emulphor-620 (Rhone-Poulenc, Inc., Princeton, NJ), and 0.9% saline at a ratio of 1:1:18. Brief halothane anesthesia was induced in animals before drug injections to minimize unnecessary stress, pain, or trauma. Animals fully recovered from anesthesia within 2 min after induction. Sham control injected animals received identical treatment.

Multiple drug treatment experiments were conducted in a manner similar to single-injection experiments with the exception that each animal received, over a period of 10 days, the entire range of drugs analyzed in the single-injection experiments. All injections throughout the treatment period were administered twice daily at approximately 10:00 AM and 6:00 PM under brief halothane anesthesia. For the multidrug treatment experiments, animals were monitored for baseline seizure frequency and duration for 1.5 days before initiation of the dosing regimen. Animals were then consecutively treated with vehicle solution for 1 day, *S*(−)WIN55,212 (5 mg/kg i.p.) for 1.5 days, *R*(+)WIN55,212 (5 mg/kg i.p.) for 2.5 days, a 2-day drug-free period during which the animals received no injections, SR141716A (10 mg/kg i.p.) for 1 day, and finally a 1-day drug-free period. Only generalized tonic-clonic seizures were counted and later confirmed by an observer blind to experimental treatment. Methylscopolamine nitrate, pilocarpine nitrate, *S*(−)WIN55,212, *R*(+)WIN55,212, phenytoin, and diazepam were purchased from Sigma-Aldrich (St. Louis, MO). SR141716A and THC were supplied through the National Institute on Drug Abuse Chemical Synthesis and Drug Supply Program.

Measurement of Hippocampal 2-AG Levels. Pilocarpine was used to acutely induce seizure activity in naive, male, Sprague-Dawley rats weighing 200 to 250 g. In these studies, animals were injected with scopolamine and 375 mg/kg i.p. pilocarpine as described under *Pilocarpine-Induced Status Epilepticus* and were sacrificed at 15 min post onset of status epilepticus. Age-matched, sham control animals were also sacrificed. Hippocampi were immediately dissected and flash frozen in liquid nitrogen. 2-AG was isolated and detected using high-performance liquid chromatography-mass spec-

troscopy according to previously published methods (Di Marzo et al., 2000).

Western Blot Protocol. Gel electrophoresis was carried out on rat hippocampal neuronal membrane preparations from 1 year after SE, epileptic, and age-matched, sham-treated animals. After monitoring of epileptic animals to verify seizure activity, the rats were sacrificed, and hippocampal tissue was harvested on ice. Hippocampi were homogenized in 50 mM Tris, pH 7.5, 6 mM EGTA, 320 mM sucrose, 1 mM dithiothreitol, and 0.3 mM phenylmethylsulfonyl fluoride, and neuronal membranes were isolated by centrifugation (Morris et al., 2001). Before electrophoresis, membrane samples were thawed on ice, and protein concentration per sample was calculated using the MicroBradford reagent system (Bio-Rad, Hercules, CA) quantified using a UV-2101PC ultraviolet spectrophotometer (Shimadzu, Kyoto, Japan). Samples were balanced to 5 μg protein/gel lane and denatured in β-mercaptoethanol and loading dye buffer. Samples were then heated to 90°C for 5 min in a programmable thermal controller PTC100 (MJ Research, Watertown, MA) and allowed to cool to room temperature before loading onto a 10% Tris-HCl Ready Gel (Bio-Rad). A colorimetric molecular mass marker including standards ranging from 10 to 182 kDa (ProSieve; Cambrex Bio Science Rockland, Inc., Rockland, ME) was loaded onto the last lane of the gel to aid in determining protein size. Gels were assembled into a MiniProtean II Electrophoresis System (Bio-Rad) and resolved for 90 min at 220 V constant in Tris buffer (Bio-Rad). After electrophoresis, gels were Western blot transferred to Immobilon nylon membrane (Millipore Corp., Bedford, MA) for 2 h at 4°C using a Genie transfer apparatus (IDEA Scientific, Minneapolis, MN) at a constant 200 V. Transfer buffer consisted of Tris-glycine buffer containing 10% methanol. After transfer, the Western blot was stored in phosphate-buffered saline at 4°C overnight. Gels were stained for protein and quantitated for microtubule-associated protein 2 and tubulin protein levels as described previously (Morris et al., 2001).

Immunostaining of the Western blot was performed by first blocking the membrane in buffer composed of 3% blotting grade blocker (Bio-Rad) and 0.05% Tween 20 in phosphate-buffered saline for 45 min at room temperature. Rabbit (polyclonal) anti-cannabinoid CB_1 receptor unconjugated primary antibody (Biosource International, Camarillo, CA) was added to the blocking solution at a concentration of 1μg/ml, and the membrane was incubated for 90 min at room temperature. After primary antibody incubation, the membrane was washed for a total of 15 min (three times for 5 min each) in phosphate-buffered saline. The membrane was then reblocked in fresh blocking buffer for 30 min. Anti-rabbit IgG-horseradish peroxidase-conjugated antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) was then added to the blocking solution in a 1:2000 dilution, and the membrane was incubated for a final 45 min. Western blots were washed (three times for 5 min each) in PBS and incubated for 5 min in SuperSignal (Pierce Chemical, Rockford, IL) for enhanced chemiluminescent analysis. Chemiluminescent images were visualized using Kodak X-Omat Blue XB-1 X-ray film (Eastman Kodak, Rochester, NY) and developed using a Kodak M35A X-Omat Processor (Eastman Kodak). Film images were digitized using a gel scanner and analyzed by computer-assisted densitometry (Amersham Biosciences Inc., Piscataway, NJ). Using the molecular mass marker as reference, the protein band was determined to correspond to a mass of 64 kDa.

Immunohistochemistry. Four epileptic and four control rats were transcardially perfused with isotonic saline, and brains were quick frozen and stored at −80°C in embedding compound (Sakura Inc., Japan). Cryostat sections (10 μM) were fixed in acetone and prepared for immunostaining using established techniques (Pettit et al., 1998). CB_1 receptor protein immunoreactivity for each animal was evaluated using more than 15 tissue sections. Briefly, tissue sections were blocked in bovine serum for 1 h and then incubated with CB_1 antiserum at 5.0 μg/ml for 1 h at room temperature. Tissue slices were then washed in PBS (three washes, each for 5 min), followed by biotinylated anti-rabbit IgG at 1:200 dilution for 30 min at room temperature. After again washing in PBS for 15 min, CB_1 receptor immunoreactivity was visualized by exposure to avidin-biotin complex and 3-3′-diaminobenzidine (Vector Laboratories, Burlingame, CA). Adjacent tissue sections were evaluated morphologically using Nissl stain. Stained tissue sections were evaluated using a binocular microscope (Olympus America Inc., Melville, NY) and were photographed using a digital camera (Olympus America Inc.). Images were analyzed using Analysis software (Soft Imaging System Corp., Lakewood, CO).

Statistical Analyses. Results are given as means ± S.E.M. Statistical comparisons were made using SigmaStat software (SPSS Science, Chicago, IL). The Student's t test, one-way analysis of variance (ANOVA), and the repeated measures (RM) one-way ANOVA in conjunction with the post hoc Tukey test were used where appropriate. Graphs were generated using Origin 6.1 software (OriginLab Corp, Northampton, MA).

Results

Modulation of the CB_1 Receptor Alters Seizure Frequency in the Rat Pilocarpine Model of Epilepsy. Epileptic rats manifested an average of 3.0 (± 0.9) seizures per 10-h period. Representative control and epileptic seizure EEG patterns are shown in Fig. 1. Control animals never manifested EEG or behavioral seizures. Administration of the CB_1 receptor agonists R(+)WIN55,212 (5 mg/kg i.p.) (Fig. 1 and 2A) and THC (30 mg/kg i.p.) (Fig. 2A), the primary psychoactive active compound in marijuana, completely terminated both behavioral and electrographic seizures in this refractory seizure model ($p \leq 0.05$). R(+)WIN55,212 and THC began having anticonvulsant effects at 0.5 and 5 mg/kg i.p., respectively. The dose response evaluation of these compounds revealed approximate ED_{50} values of 1 mg/kg i.p. for R(+)WIN55,212 and 15 mg/kg i.p. for THC. The concentrations of THC and R(+)WIN55,212 required to inhibit seizures in this model were similar in effect and dose to their ability to inhibit maximal electric shock-induced seizures (Wallace et al., 2001), and this concentration of THC has been shown to have anticonvulsant effects in other seizure models (Wada et al., 1975; Karler and Turkanis, 1980; Colasanti et al., 1982). At the maximal anticonvulsant doses of THC and R(+)WIN55,212, the animals were not significantly sedated and were alert enough to be able to move freely in the their cages. These ED_{50} values are below the ED_{50} values for R(+)WIN55,212 and THC in decreasing spontaneous activity and similar to the concentrations used to cause hypothermia and analgesia (Wallace et al., 2001). Thus, the anticonvulsant effects of THC and R(+)WIN55,212 are in the same

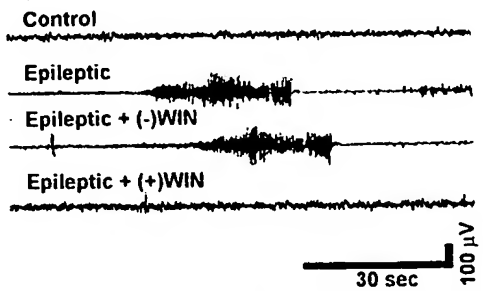

Fig. 1. The effects of CB_1 receptor modulation on epileptiform activity in control and epileptic animals. Representative EEG recordings of control, epileptic, S(−)WIN55,212-, [(−)WIN]-treated (5 mg/kg i.p.) epileptic animals and R[(+)WIN]55,212-, [(+)WIN]-treated (5 mg/kg i.p.) epileptic animals. Treatment with R(+)WIN55,212 (5 mg/kg i.p.) completely abolished seizure activity.

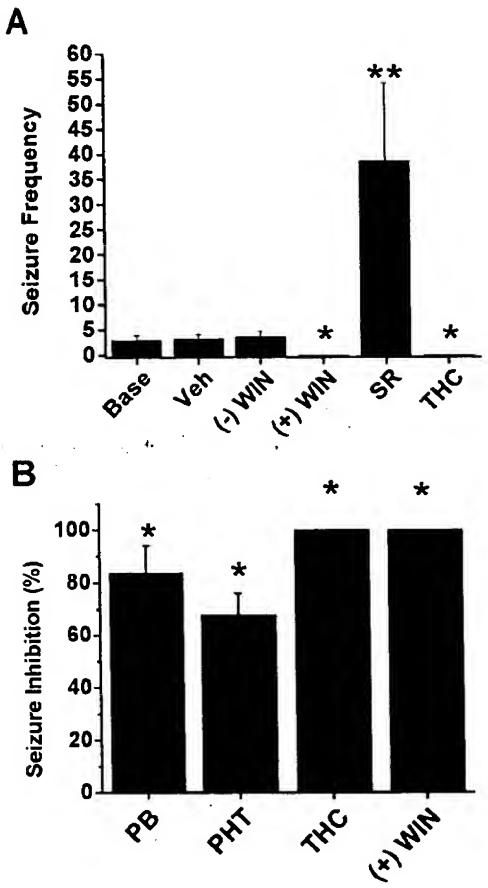

Fig. 2. The effects of CB_1 receptor activation and blockade on the seizure frequency of epileptic rats demonstrated in single-injection experiments. A, seizure frequency per 10 h for baseline (base) and treatment with vehicle (Veh), *S*(−)WIN55,212, [(−)WIN] (5 mg/kg i.p.), *R*(+)WIN55,212, [(+)WIN] (5 mg/kg i.p.), SR141716A (SR) (10 mg/kg i.p.), and THC (30 mg/kg i.p.). Data represent mean ± S.E. (n = 6 per drug treatment; ⋆, $p \leq 0.05$; ⋆⋆, $p \leq 0.001$). B, inhibition of seizure activity at high therapeutic concentrations (Morris et al., 2001) by the anticonvulsants phenobarbital (PB; 40 mg/kg i.p.) and phenytoin (PHT; 100 mg/kg i.p.) and the cannabinoids THC (30 mg/kg i.p.) and *R*(+)WIN55,212, (+)WIN (5 mg/kg i.p.) (n = 6 per drug treatment; ⋆, $p \leq 0.01$ in comparison with epileptic animals). Only THC and (+)WIN completely abolished seizure activity. These single-injection experiments directly evaluated the effects of each agent on seizure frequency in multiple animals.

concentration range of some of the other physiological effects of the cannabinoids but below sedative concentrations. The inactive isomer, *S*(−)WIN55,212 (5 mg/kg i.p.), and drug vehicle alone had no effect on seizure frequency (Fig. 1 and 2A). The enantioselectivity of the anticonvulsant effect of *R*(+)WIN55,212 strongly indicates that this compound is acting via a CB_1 receptor-specific mechanism. Maximally effective doses that produce high therapeutic blood levels of phenobarbital (40 mg/kg i.p.) and phenytoin (100 mg/kg i.p.), well established anticonvulsants, do not completely inhibit seizure activity in this model of refractory epilepsy (Leite and Cavalheiro, 1995; Morris et al., 2001). Because of the inability of these standard anticonvulsants to completely block seizures when used in high therapeutic levels in this model, the pilocarpine model of epilepsy is considered a model of refractory or difficult-to-control seizures with conventional anticonvulsant agents. The cannabinoids were very effective anticonvulsants in this model at a concentration that did not produce sedation but completely abolished seizures. Phenobarbital and phenytoin at very high concentrations were not as effective. Thus, treatment of animals with phenobarbital and phenytoin was less efficacious than cannabinoids in preventing behavioral and electrographic seizures produced in this model (Fig. 2B), indicating that cannabinoids may offer unique advantages in treating seizures refractory to currently prescribed anticonvulsants.

Seizure characteristics can vary between animals, a limiting factor in the interpretation of data. Therefore, to increase the statistical power of the study, we tested the effects of cannabinoids on seizure frequency and duration by systematically treating a group of eight epileptic animals over a 10-day period with a multiple drug treatment regimen. This paradigm consisted of baseline (1.5 days), drug vehicle (1 day), *S*(−)WIN55,212 (5 mg/kg i.p.) (1.5 days), *R*(+)WIN55,212 (5 mg/kg i.p.) (2.5 days) followed by a drug-free period (2 days), SR141716A (10 mg/kg i.p.) (1 day), and ending with a drug-free period (1.5 days) (Fig. 3A). Seizure frequency for these epileptic animals during baseline recording ranged between one and three per 12-h recording interval. Treatment with vehicle or the inactive isomer *S*(−)WIN55,212 had no statistically significant effect on seizure frequency (Fig. 3, A and B). Conversely, treatment with *R*(+)WIN55,212 abolished seizures in all eight animals used in this treatment paradigm (Fig. 3B; $p \leq 0.05$). During the drug-free period after treatment with *R*(+)WIN55,212, seizure frequency increased slightly above baseline (Fig. 3, A and B). The observed increase in seizure frequency after cannabinoid cessation is consistent with the withdrawal phenomenon and rebound hyperexcitability described in other behavioral studies (Karler et al., 1986). However, this rebound effect was transient, with seizure frequency in most animals returning to levels similar to baseline by the later half of the second day of drug withdrawal. On day 9, we administered a single injection of SR141716A that produced a significant but reversible increase in seizure frequency compared with baseline or the drug withdrawal seizure frequency (Fig. 3, A and B), supporting the hypothesis that endogenous cannabinoids act tonically to dampen neuronal hyperexcitability. The effect of SR141716A was significantly elevated above the baseline and the rebound periods. In addition, SR141716A treatment alone clearly produced increased seizure frequency in epileptic animals (Fig. 2A). The multiple treatment experiments demonstrated a tolerance effect. Long-term administration of cannabinoids affected seizure frequency when the cannabinoids were discontinued. Further study of the tolerance effect of the cannabinoids on the CB_1 receptor in epileptic animals is an important area for further investigation but is beyond the scope of the present study.

Antagonism of the CB_1 Receptor Significantly Increases Seizure Frequency and Duration. Administration of SR141716A to epileptic rats resulted in a statistically significant increase in seizure frequency (Fig. 2A and 3B; $p \leq 0.01$). Figure 4 compares 60 min of continuous EEG recording before and after SR141716A treatment in an epileptic animal. This EEG recording is representative of the increased seizure frequency observed in all animals treated with SR141716A. Several SR141716A-treated animals developed SE, a severe prolonged seizure condition associated with a high morbidity and mortality (DeLorenzo et al., 1996). In these animals, EEG seizure activity was nearly continuous,

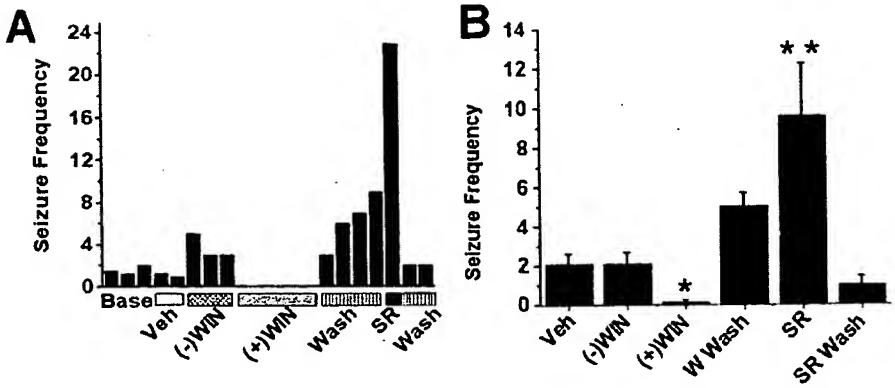

Fig. 3. The effects of CB_1 receptor activation and blockade on the seizure frequency in eight epileptic rats sequentially treated with a multiple drug regimen that includes a CB_1 receptor agonist and antagonist. These experiments evaluate the effects of each drug in comparison with the other drugs in the same animal. A, seizure frequency per 12 h in a representative epileptic animal after consecutive administration of vehicle, *S*(−)WIN55,212, (−)WIN (5 mg/kg i.p.), *R*(+)WIN55,212, (+)WIN (5 mg/kg i.p.), *R*(+)WIN55,212 (5 mg/kg i.p.) washout (W wash), SR141716A, SR (10 mg/kg i.p.), and SR141716A washout (SR wash). Bars represent the number of seizures observed in a representative epileptic animal for each 12-h monitoring period. B, mean seizure frequency (per 12 h) of eight epileptic animals treated with the same drug regimen shown in Fig. 3A. This figure presents the mean data for the multiple drug experiments for each experimental condition and analyzes the data statistically. Data represent the mean ± S.E. (seizures per 12 h) (n = 8; RM ANOVA; *, $p \leq 0.05$; **, $p \leq 0.01$).

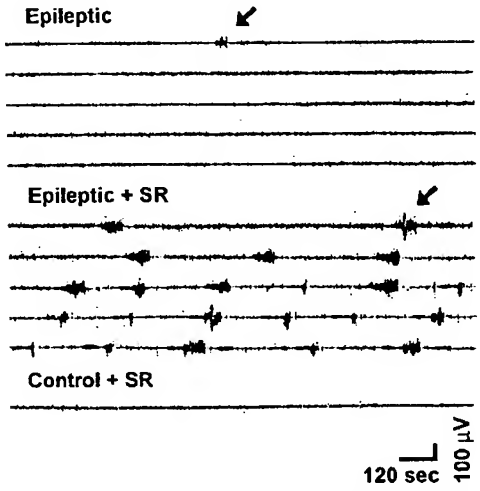

Fig. 4. Antagonism of the CB_1 receptor by SR141716A (10 mg/kg i.p.) caused increased seizure frequency and produced status epilepticus in some animals. The data represent EEG and behavioral seizures observed over the 1-h recording period for epileptic and epileptic + SR conditions. These recordings represent continuous EEG recordings from an epileptic rat 60 min before and 60 min after treatment with SR141716A. Arrows represent individual seizures. The representative EEG recording from an epileptic animal manifested one spontaneous recurrent seizure in the 1 h of recording. SR treatment in epileptic animals caused a marked increase in seizure frequency. During the numerous seizures shown for SR141716A treatment in the 1-h recording, the animal was not responsive in between seizures for more than 30 min. Thus, SR141716A produced status epilepticus in this animal, employing the standard definition of SE that includes intermittent seizure activity lasting for more than 30 min without regaining consciousness between seizures. The Control + SR representative EEG recording demonstrates that treatment of control (nonepileptic) animals with SR141716A did not produce seizure activity.

and animals were unresponsive to external stimuli with loss of righting reflex for 30 min or more. SR141716A (10 mg/kg i.p.) has been shown to inhibit the anticonvulsant effects of cannabinoids and endocannabinoids (Wallace et al., 2001, 2002). SR141716A was also effective in blocking the anticonvulsant effects of THC and *R*(+)WIN55,212 at 5 mg/kg i.p. We choose to use the higher concentration of SR141716A to obtain a clear antagonist effect. In addition, SR141716A (10 mg/kg i.p.) did not induce seizures in control animals (Fig. 4). The effect of SR141716A was only observed in the epileptic animals, and this compound did not cause hyperexcitability in control or naive animals.

To further evaluate the role of endogenous CB_1 receptor activation on seizure termination, we quantified the duration of individual seizure events within each drug treatment period (Fig. 5, A and B). In all animals monitored, EEG seizures directly coincided with behavioral seizures observed on video recording. During vehicle and *S*(−)WIN55,212 treatments, seizure duration was not significantly altered from baseline. Treatment with the CB_1 receptor antagonist, SR141716A, caused a significant increase in seizure duration ($p \leq 0.01$; Fig. 5, A and B). Prolongation of seizure discharge by SR141716A is apparent in the EEG patterns of representative seizure events (Fig. 5A).

Hippocampal Levels of 2-AG Increase during Seizure Activity. 2-AG synthesis occurs during neuronal depolarization in a Ca^{2+}-dependent manner (Stella et al., 1997). Sustained neuronal depolarization and elevated intracellular Ca^{2+} are known to accompany seizure activity (Raza et al., 2001). We have previously shown that endogenous cannabinoids are anticonvulsant and this anticonvulsant activity of the endocannabinoids could be blocked by SR141716A (Wallace et al., 2002). If the endogenous cannabinoid system contributes to epileptic seizure termination, seizure activity in an intact animal would be expected to increase synthesis of endogenous cannabinoids. The hippocampal brain region is a locus of epileptic seizure activity (Lothman et al., 1991); therefore, we sought to determine the effect of status epilepticus on hippocampal levels of 2-AG. Pilocarpine-injected animals were sacrificed after 15 min of pilocarpine-induced seizure activity along with sham controls. Levels of 2-AG in hippocampal extracts were determined according to previously published methods (Di Marzo et al., 2000). In acutely seizing animals, endogenous 2-AG levels were significantly increased compared with controls (Fig. 5C; $p \leq 0.05$). The data demonstrate that a single pilocarpine-induced seizure can increase the level of the endogenous cannabinoid 2-AG in hippocampal tissue. We have also demonstrated that

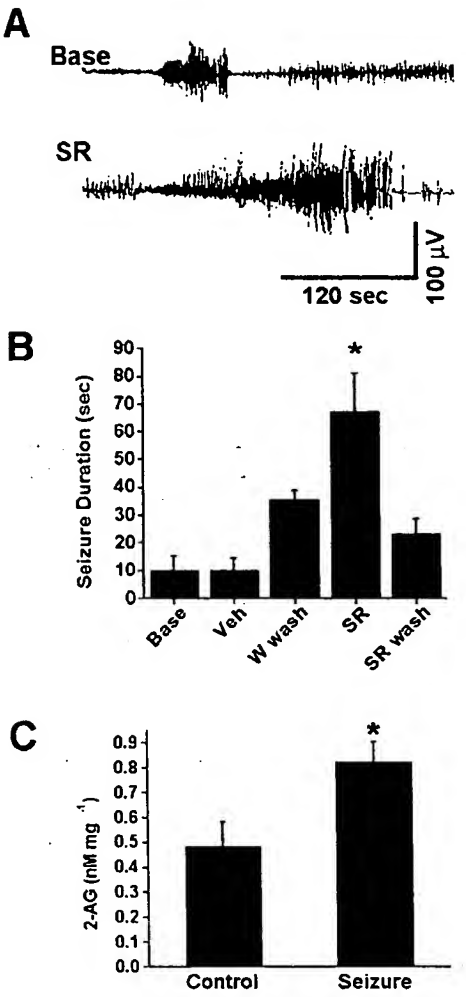

Fig. 5. CB_1 receptor-dependent regulation of seizure duration in epileptic rats. A, a representative EEG recording of a seizure in an epileptic (Base) and an epileptic animal treated with SR141716A (SR) (10 mg/kg i.p.) demonstrating increased seizure duration produced by SR. B, mean seizure duration for the treatments shown in Fig. 3B. Data represent the mean ± S.E. (n = 8 animals; RM ANOVA; ⋆, $p \leq 0.01$). C, hippocampal endogenous 2-AG levels in control and seizure animals (15 min after seizure onset). The data represent the mean ± S.E. (n = 7; ⋆, $p \leq 0.01$; Student's t test)

SR141716A (10 mg/kg i.p.) inhibited the anticonvulsant effects of endocannabinoids (Wallace et al., 2002).

Increased Hippocampal CB_1 Receptor Expression in Epileptic Rats. Because CB_1 receptor activation was shown to alter seizure frequency and duration, we sought to evaluate possible changes in CB_1 receptor expression in the hippocampi of epileptic animals. Using Western blot analyses, we compared sham control with epileptic hippocampal neuronal membranes and found a significant increase in epileptic brains of the expression of the 64-kDa molecular mass CB_1 receptor protein (Cichewicz et al., 2001) (Fig. 6A). Quantification of the bands shown in Fig. 6A revealed that expression of this protein was increased 183% in the hippocampi of epileptic rats compared with sham-treated animals (Fig. 6B; $p \leq 0.01$), indicating that a long-term plasticity change in the expression of the CB_1 receptor occurs with epilepsy. Western blot values were further corrected using the internal membrane protein markers tubulin and microtubule-associated protein 2 proteins (data not shown). After correcting cannabinoid protein levels to internal protein standards, we still observed a significant increase in the CB_1 receptor expression in epileptic animals (data not shown).

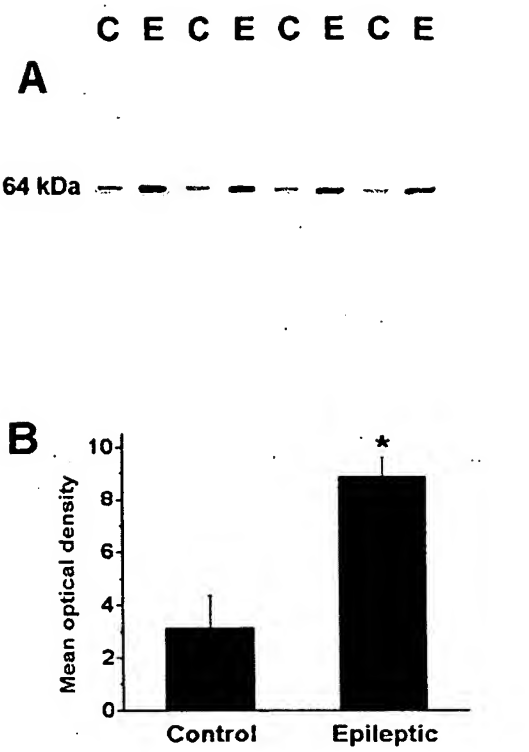

Fig. 6. CB_1 receptor expression is increased in hippocampal neuronal membranes of epileptic rats. A, representative Western blot of sham control (C) and epileptic (E) hippocampal neuronal membranes. The band visualized corresponded to the 64-kDa CB_1 receptor protein (Cichewicz et al., 2001). B, mean optical densities (arbitrary units) of CB_1 receptor protein expression in hippocampal membranes of epileptic and sham control rats (n = 6; ⋆, $p \leq 0.01$; Student's t test). Epileptic CB_1 receptor protein was increased by 183%.

To evaluate the anatomical distribution of this increase in CB_1 receptor expression in epileptic brains, we conducted immunohistochemical staining of CB_1 receptor protein on coronal hippocampal sections using established techniques (Pettit et al., 1998). Figure 7, A and B, shows representative patterns of cellular Nissl staining in sham control and epileptic hippocampi. No apparent changes in hippocampal morphology were observed in epileptic versus sham control animals, with the exception of minimal cell loss in the CA1 region (less than 10%), as described previously (Rice and DeLorenzo, 1998). Representative pseudocolor-enhanced images of CB_1 receptor protein staining of epileptic and sham control hippocampal sections illustrate a dramatic increase in CB_1 receptor expression in epileptic hippocampi (Fig. 7, C and D). CB_1 receptor protein staining was most dramatically increased in the CA1 through CA3 regions of the hippocampus, with the highest increase localized to the dendritic synaptic areas of CA2 and CA3 (Fig. 7, E-G). The dentate gyrus did not show a corresponding increase in CB_1 receptor expression.

Discussion

In the present study, we report that the endogenous cannabinoid system plays a critical role in modulating seizure activity in epilepsy. Both cannabinoids, THC and

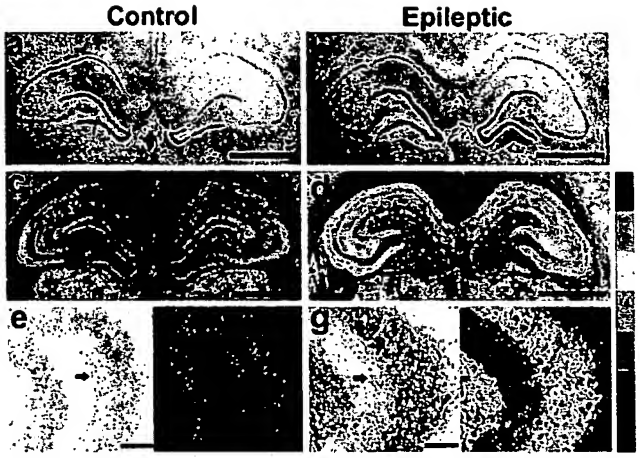

Fig. 7. Immunohistochemical detection of CB_1 receptor expression in control and epileptic hippocampi. Representative Nissl staining of control (A) and epileptic (B) sections. Representative pseudocolor-enhanced immunohistochemical staining of the CB_1 receptor protein in control (C) and epileptic (D) sections. The increase in CB_1 receptor protein expression observed in epileptic hippocampi was representative of four epileptic versus four control animals, 15 tissue sections per animal. High magnification of CB_1 receptor immunoreactivity of sham control (E) and epileptic (G) hippocampal formation demonstrated increased staining in the dendritic fields of the CA2 and CA3 regions of epileptic animals. Arrows indicate the location of the CA2 through CA3 pyramidal neurons. High magnification of pseudocolor-enhanced images of sham control (F) and epileptic (H) CA2 and CA3 regions. Bars in A through D represent 2 mm. Bars in E through H represent 200 μm. Red, highest level on color scale. The results shown are representative of several experiments.

R(+)WIN55,212, were anticonvulsant in the rat pilocarpine model of acquired, refractory epilepsy. Antagonism of the CB_1 receptor by SR141716A caused a marked increase in seizure frequency and duration, indicating that endogenous activity of the CB_1 receptor strongly influences seizure activity. By antagonizing the CB_1 receptor, we blocked its activation by endogenous cannabinoids and thereby elicited a sustained seizure response that often resembled the clinical phenomenon status epilepticus. The rise in endogenous 2-AG levels that occurred during short-term, pilocarpine-induced seizure demonstrates that seizure activity can increase the levels of 2-AG and further suggests a modulatory role for endogenous cannabinoids in epilepsy. Additionally, using Western blot and immunohistochemical techniques, we determined that hippocampal CB_1 receptor expression in epileptic animals was significantly increased over sham controls. These changes were primarily observed in the CA1 through CA3 regions of the hippocampus, indicating that plasticity of the endogenous cannabinoid system occurs in this brain region in response to epilepsy. These data provide evidence that the CB_1 receptor and the endogenous cannabinoid system play a critical role in dampening epileptic neuroexcitation. Piomelli's group (Rodriguez de Fonseca et al., 2001) has demonstrated that it is possible to trigger physiological effects of cannabinoids without producing the unwanted behavioral or psychoactive effects of these compounds. Thus, the development of anticonvulsant cannabinoids that do not produce unwanted side effects is an important area for further research to develop novel therapeutic agents to treat intractable seizures.

Epilepsy Increases Hippocampal CB_1 Receptor Expression. The findings presented in this study demonstrate that a significant change in CB_1 receptor expression occurs with the epileptic phenotype. The animals used in this study had been epileptic for nearly 1 year, indicating that this change in CB_1 receptor expression is prolonged and probably permanent. CB_1 receptor expression has also been shown to increase in an animal model of stroke (Jin et al., 2000), a condition, like epilepsy, that is associated with excessive glutamate release and the development of seizures. The observation that the cannabinoid receptor is up-regulated in ischemia and epilepsy implies a compensatory role for the receptor in mitigating excitotoxicity. In light of the anticonvulsant effect of both *R*(+)WIN55,212 and THC, as well as the proconvulsant action of the CB_1 receptor antagonist in this epilepsy model, we propose that the increase in CB_1 receptor expression displayed in epileptic brains was a compensatory rather than a causal factor of seizure manifestation and served to dampen seizure activity. The increased CB_1 receptor expression displayed in the hippocampi of epileptic animals was regionally specific, occurring in the CA dendritic field and not in the dentate gyrus. This increase in CB_1 receptor expression was demonstrated up to 1 year after the induction of epilepsy and thus demonstrates a long-lasting or permanent plasticity change in the brain that may play a role in the pathophysiology of epilepsy. The functional relevance of this differential increase in CB_1 receptor expression may be revealed by further study.

Anticonvulsant Action of Cannabinoids. Recent discoveries in the cannabinoid field have demonstrated that cannabinoids ameliorate symptoms associated with neuronal hyperexcitability. In models of multiple sclerosis (Baker et al., 2000) and Huntington's disease (Lastres-Becker et al., 2002), CB_1 receptor activation significantly reduced spasticity and tremor, and exogenous application of 2-AG has been shown to be neuroprotective after traumatic brain injury (Panikashvili et al., 2001). Furthermore, in in vitro and in vivo studies of ischemia, cannabinoids significantly decreased excitotoxic neuronal cell death that resulted from excessive glutamatergic transmission (Abood et al., 2001). These cannabinoid actions are believed to involve attenuation of glutamate release. At the molecular level, the anticonvulsant mechanism of cannabinoids is unknown. However, because modulation of presynaptic neurotransmitter release is believed to be a primary result of CB_1 receptor activation, we believe that this mechanism may underlie cannabinoid anticonvulsant properties. CB_1 receptor activation is known to decrease calcium influx through N- and P/Q-type Ca^{2+} channels (Mackie and Hille, 1992), the result of which is decreased Ca^{2+}-dependent glutamate release. Glutamate is the primary excitatory neurotransmitter of the central nervous system. Although critical for normal neurotransmission, elevated levels of glutamate are associated with excitotoxicity and excessive glutamatergic transmission is a hallmark of epilepsy (Lothman et al., 1991). With elevated levels of glutamate detected in epileptic tissue (Lothman et al., 1991), decreased release of this neurotransmitter would be a logical cannabinoid anticonvulsant mechanism. CB_1 receptor activation also increases the conductance of presynaptic A-type (Hampson et al., 1995) and G-protein-coupled inward rectifying K^+ channels (Mackie et al., 1995). Increased K^+ channel permeability attenuates neuronal bursting and stabilizes membrane potential, additional factors that would contribute to decreased epileptiform discharge. Preliminary data from our group indicates that CB_1

knockout animals have spontaneous seizures, further suggesting an endogenous role for the CB_1 receptor in controlling neuronal excitability.

CB_1 receptor activation has also been shown to decrease GABAergic function in the hippocampus. In particular, endogenous cannabinoids are believed to be retrograde mediators of depolarization-induced suppression of inhibition (DSI) (Wilson and Nicoll, 2001). The overall effect of DSI at the synapse is disinhibition of the postsynaptic neuron and, therefore, facilitation of excitatory transmission. In light of the increased neuronal excitability that may result from this action, decreased GABAergic tone most probably does not mediate the anticonvulsant mechanism of cannabinoids. However, Cohen et al. (2002) recently demonstrated that the GABAergic system, normally an inhibitory neurotransmitter, can become a depolarizing force capable of synchronizing abnormal bursting in human epileptic, temporal lobe, brain slice preparations. If this phenomenon were to occur within the brains of animals with pilocarpine-induced epilepsy, then a cannabinoid-mediated decrease in GABAergic tone may indeed be anticonvulsant.

A more probable explanation for the anticonvulsant action of cannabinoids lies in the possibility that the pathology of epilepsy causes a compensatory shift to occur in the balance between CB_1 receptor-mediated inhibition of presynaptic glutamate and GABA release. In support of this, recent studies have shown that, in a manner similar to DSI, depolarization-induced suppression of excitation can be induced in hippocampal tissue (Ohno-Shosaku et al., 2002). The induction of this phenomenon was dependent on the sensitivity of the presynaptic neuron to cannabinoids as well as the duration of postsynaptic depolarization. With extended depolarization, the result of CB_1 receptor activation was a shift from DSI to depolarization-induced suppression of excitation. Therefore, the extended neuronal depolarization of an epileptiform discharge may cause a switch from suppression of GABA release to suppression of glutamate release.

Synthesis of the endogenous cannabinoid 2-AG is believed to occur in a calcium-dependent, "on-demand" fashion from arachidonic acid-enriched membrane phospholipids. During a seizure, elevated intracellular Ca^{2+} results from prolonged neuronal depolarization (Raza et al., 2001). Because increased hippocampal levels of 2-AG were detected 15 min into a seizure and CB_1 receptor antagonism resulted in prolonged seizure duration, we believe that seizure-induced increases in intracellular calcium result in the de novo synthesis of endogenous cannabinoids that then bind the CB_1 receptor to terminate seizure discharge, forming a negative feedback loop. This increase in 2-AG occurred in comparison with sham animals and was shown to be dependent on seizure activity and not manipulation of the animals or drug-specific effects. Additional evidence of compensatory endogenous cannabinoid release during seizure activity is provided by studies that show elevated 2-AG after injection of the chemoconvulsant picrotoxin (Sugiura et al., 2000). 2-AG is known to bind the CB_1 receptor with high affinity in a manner that is blocked by coadministration of SR141716A.

Several factors in addition to increased production of 2-AG could explain seizure-induced increase in the levels of this compound. Alternatively, increased 2-AG levels during seizures may be the result of decreased function of the fatty acid amidohydrolase enzyme that is known to be responsible for the catalysis of the compound. Increased receptor sensitivity and reducing cannabinoid catabolism during seizure activity could also account for the net increase in 2-AG observed after seizure activity. Further study may reveal which mechanism generates this increase in hippocampal 2-AG.

Therapeutic Implications for Cannabinoids in the Treatment of Epilepsy. Seizures in patients with refractory, partial-complex epilepsy can be difficult to control despite the use of currently available anticonvulsant medications and surgical interventions. Therefore, there is a clear need for the development of more effective anticonvulsant agents. Some epilepsy patients, seeking alternative treatments, have perceived improvement with marijuana (Consroe et al., 1975). This has prompted several countries to consider the legalization of marijuana for epilepsy treatment (National Institutes of Health, 1997; R. v. Parker, 1997; House of Lords Select Committee on Science and Technology, 1998). The pilocarpine model represents a refractory epileptic condition that is not readily treated by conventional anticonvulsants (Leite and Cavalheiro, 1995; Morris et al., 2001). Our results demonstrate that activation of the CB_1 receptor by cannabinoid drugs and possibly endogenous ligands significantly alters seizure activity and is more effective than conventional anticonvulsants in treating the refractory seizures produced in the pilocarpine model. Although the dose dependence and long-term effects of cannabinoid administration on epilepsy must be further investigated, the results presented here provide evidence that warrants a comprehensive assessment of cannabinoid use in the control of refractory epilepsy via the use of animal models and placebo-controlled clinical trials. Although the psychoactive side effects of cannabinoids make their use in the treatment of epilepsy impractical, understanding the mechanisms of endogenous cannabinoid-mediated anticonvulsant action may lead to the development of novel compounds that do not manifest behavioral toxicity. Further investigation of the cannabinoid anticonvulsant phenomenon may illuminate novel therapeutic targets for the treatment of temporal lobe epilepsy as well as more clearly define the physiological function of the endogenous cannabinoid system in brain.

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References

Abood ME, Rizvi G, Sallapudi N, and McAllister SD (2001) Activation of the CB_1 cannabinoid receptor protects cultured mouse spinal neurons against excitotoxicity. *Neurosci Lett* 309:197–201.

Baker D, Pryce G, Croxford JL, Brown P, Pertwee RG, Huffman JW, and Layward L (2000) Cannabinoids control spasticity and tremor in a multiple sclerosis model. *Nature (Lond)* 404:84–87.

Cichewicz DL, Haller VL, and Welch SP (2001) Changes in opioid and cannabinoid receptor protein following short-term combination treatment with Δ^9-tetrahydrocannabinol and morphine. *J Pharmacol Exp Ther* 297:121–127.

Cohen I, Navarro V, Clemenceau S, Baulac M, and Miles R (2002) On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science (Wash DC)* 298:1418–1421.

Colasanti BK, Lindamood C 3rd, and Craig CR (1982) Effects of marihuana cannabinoids on seizure activity in cobalt-epileptic rats. *Pharmacol Biochem Behav* 16:573–578.

Consroe PF, Wood GC, and Buchsbaum H (1975) Anticonvulsant nature of marihuana smoking. *J Am Med Assoc* 234:306–307.

DeLorenzo RJ, Hauser WA, Towne AR, Boggs JG, Pellock JM, Penberthy L, Garnett L, Fortner CA, and Ko D (1996) A prospective, population-based epidemiologic study of status epilepticus in Richmond, Virginia. *Neurology* 46:1029–1035.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D,

Mandelbaum A, Etinger A, and Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science (Wash DC)* 258:1946–1949.

Di Marzo V, Breivogel CS, Tao Q, Bridgen DT, Razdan RK, Zimmer AM, Zimmer A, and Martin BR (2000) Levels, metabolism and pharmacological activity of anandamide in CB_1 cannabinoid receptor knockout mice: evidence for non-CB_1, non-CB_2 receptor-mediated actions of anandamide in mouse brain. *J Neurochem* 75: 2434–2444.

Hampson RE, Evans GJ, Mu J, Zhuang SY, King VC, Childers SR, and Deadwyler SA (1995) Role of cyclic AMP dependent protein kinase in cannabinoid receptor modulation of potassium "A-current" in cultured rat hippocampal neurons. *Life Sci* 56:2081–2088.

Hauser WA and Hesdorffer DC (1990) *Epilepsy: Frequency, Causes and Consequences*. Demos, New York.

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, and Rice KC (1990) Cannabinoid receptor localization in brain. *Proc Natl Acad Sci USA* 87:1932–1936.

House of Lords Select Committee on Science and Technology (1998) *Cannabis: The Scientific and Medical Evidence*, chapter 5, section 5.3, London. Available at: http://www.publications.parliament.uk/pa/ld199798/ldselect/ldsctech/151/15106.htm

Jin KL, Mao XO, Goldsmith PC, and Greenberg DA (2000) CB_1 cannabinoid receptor induction in experimental stroke. *Ann Neurol* 48:257–261.

Karler R, Calder LD, and Turkanis SA (1986) Prolonged CNS hyperexcitability in mice after a single exposure to delta-9-tetrahydrocannabinol. *Neuropharmacology* 25:441–446.

Karler R, Cely W, and Turkanis SA (1974) Anticonvulsant properties of delta 9-tetrahydrocannabinol and other cannabinoids. *Life Sci* 15:931–947.

Karler R and Turkanis SA (1980) Subacute cannabinoid treatment: anticonvulsant activity and withdrawal excitability in mice. *Br J Pharmacol* 68:479–484.

Lastres-Becker I, Hansen HH, Berrendero F, De Miguel R, Perez-Rosado A, Manzanares J, Ramos JA, and Fernandez-Ruiz J (2002) Alleviation of motor hyperactivity and neurochemical deficits by endocannabinoid uptake inhibition in a rat model of Huntington's disease. *Synapse* 44:23–35.

Leite JP and Cavalheiro EA (1995) Effects of conventional antiepileptic drugs in a model of spontaneous recurrent seizures in rats. *Epilepsy Res* 20:93–104.

Lothman EW, Bertram EH, and Stringer JL (1991) Functional anatomy of hippocampal seizures. *Prog Neurobiol* 37:1–82.

Mackie K and Hille B (1992) Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. *Proc Natl Acad Sci USA* 89:3825–3829.

Mackie K, Lai Y, Westenbroek R, and Mitchell R (1995) Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *J Neurosci* 15:6552–6561.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, et al. (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* 50:83–90.

Mello LE, Cavalheiro EA, Tan AM, Kupfer WR, Pretorius JK, Babb TL, and Finch DM (1993) Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. *Epilepsia* 34:985–995.

Morris TA, Jafari N, and DeLorenzo RJ (2001) Chronic delta FosB expression and increased AP-1 transcription factor binding are associated with the long term plasticity changes in epilepsy. *Brain Res Mol Brain Res* 79:138–149.

National Institutes of Health (1997) *Workshop on the Medical Utility of Marijuana, Report to the Director*, Washington, D.C. Available at: http://www.nih.gov/news/medmarijuana/MedicalMarijuana.htm

Ohno-Shosaku T, Tsubokawa H, Mizushima I, Yoneda N, Zimmer A, and Kano M (2002) Presynaptic cannabinoid sensitivity is a major determinant of depolarization-induced retrograde suppression at hippocampal synapses. *J Neurosci* 22: 3864–3872.

Panikashvili D, Simeonidou C, Ben-Shabat S, Hanus L, Breuer A, Mechoulam R, and Shohami E (2001) An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. *Nature (Lond)* 413:527–531.

Perlin JB, Gerwin CM, Panchision DM, Vick RS, Jakoi ER, and DeLorenzo RJ (1993) Kindling produces long-lasting and selective changes in gene expression of hippocampal neurons. *Proc Natl Acad Sci USA* 90:1741–1745.

Pettit DA, Harrison MP, Olson JM, Spencer RF, and Cabral GA (1998) Immunohistochemical localization of the neural cannabinoid receptor in rat brain. *J Neurosci Res* 51:391–402.

R. v. Parker (1997), 12 C.R. (5th) 251 (Ont. Ct. Justice).

Raza M, Pal S, Rafiq A, and DeLorenzo RJ (2001) Long-term alteration of calcium homeostatic mechanisms in the pilocarpine model of temporal lobe epilepsy. *Brain Res* 903:1–12.

Rice AC and DeLorenzo RJ (1998) NMDA receptor activation during status epilepticus is required for the development of epilepsy. *Brain Res* 782:240–247.

Rodriguez de Fonseca F, Navarro M, Gomez R, Escuredo L, Nava F, Fu J, Murillo-Rodriguez E, Giuffrida A, LoVerme J, Gaetani S, et al. (2001) An anorexic lipid mediator regulated by feeding. *Nature (Lond)* 414:209–212.

Stella N, Schweitzer P, and Piomelli D (1997) A second endogenous cannabinoid that modulates long-term potentiation. *Nature (Lond)* 388:773–778.

Sugiura T, Yoshinaga N, Kondo S, Waku K, and Ishima Y (2000) Generation of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand, in picrotoxinin-administered rat brain. *Biochem Biophys Res Commun* 271:654–658.

Wada JA, Wake A, Sato M, and Corcoran ME (1975) Antiepileptic and prophylactic effects of tetrahydrocannabinols in amygdaloid kindled cats. *Epilepsia* 16:503–510.

Wallace MJ, Martin BR, and DeLorenzo RJ (2002) Evidence for a physiological role of endocannabinoids in the modulation of seizure threshold and severity. *Eur J Pharmacol* 452:295–301.

Wallace MJ, Wiley JL, Martin BR, and DeLorenzo RJ (2001) Assessment of the role of CB_1 receptors in cannabinoid anticonvulsant effects. *Eur J Pharmacol* 428:51–57.

Wilson RI and Nicoll RA (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature (Lond)* 410:588–592.

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Assessment of the role of CB_1 receptors in cannabinoid anticonvulsant effects

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Abstract

The cannabinoid CB_1 receptor has been shown to be the primary site of action for cannabinoid-induced effects on the central nervous system. Activation of this receptor has proven to dampen neurotransmission and produce an overall reduction in neuronal excitability. Cannabinoid compounds like Δ^9-tetrahydrocannabinol and cannabidiol have been shown to be anticonvulsant in maximal electroshock, a model of partial seizure with secondary generalization. However, until now, it was unknown if these anticonvulsant effects are mediated by the cannabinoid CB_1 receptor. Likewise, (*R*)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN 55,212-2), a cannabimimetic compound that has been shown to decrease hyperexcitability in cell culture models via the cannabinoid CB_1 receptor, has never been evaluated for anticonvulsant activity in an animal seizure model. We first show that the cannabinoid compounds Δ^9-tetrahydrocannabinol (ED_{50} = 42 mg/kg), cannabidiol (ED_{50} = 80 mg/kg), and WIN 55,212-2 (ED_{50} = 47 mg/kg) are anticonvulsant in maximal electroshock. We further establish, using the cannabinoid CB_1 receptor specific antagonist *N*-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride (SR141716A) (AD_{50} = 2.5 mg/kg), that the anticonvulsant effects of Δ^9-tetrahydrocannabinol and WIN 55,212-2 are cannabinoid CB_1 receptor-mediated while the anticonvulsant activity of cannabidiol is not. This study establishes a role for the cannabinoid CB_1 receptor in modulating seizure activity in a whole animal model.

Keywords: Cannabinoid; Epilepsy; Maximal electroshock; Seizure; Cannabinoid CB_1 receptor

1. Introduction

Despite marijuana's illegal status in the United States, individuals both here and abroad report its use to be therapeutic in the treatment of a variety of ailments, including epilepsy (Hollister, 1983; Adams and Martin, 1996). Approximately 1% of Americans have epilepsy and 30% of these patients are refractory to conventional antiepileptic drug treatments (Zarrelli et al., 1999). Cannabinoid compounds have been used as a natural remedy for seizures for nearly 2000 years (Adams and Martin, 1996). In 1974, Karler et al. found that Δ^9-tetrahydrocannabinol, the primary psychoactive compound in marijuana, displayed anticonvulsant properties in maximal electroshock-induced tonic–clonic convulsions (Karler et al., 1974). The non-psychoactive marijuana constituent, cannabidiol, was also shown to be protective in this seizure model (Karler et al., 1973). Since this initial research, several cannabimimetic compounds have been synthesized and evaluated in vitro for their effects on neuronal hyperexcitability. (*R*)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN 55,212-2) attenuated low-Mg^{2+} induced burst-firing in hippocampal culture (Shen and Thayer, 1999). In addition, the endogenous ligands anandamide and 2-Arachidonylglycerol were found to decrease the amplitude of stimulation-induced population spikes, as well as attenuate low-Mg^{2+}-induced epileptiform discharges in rat hippocampal slice preparation (Ameri and Simmet, 2000). The mechanism underlying this dampening of excitability is believed to involve the inhibition of presynaptic excitatory neurotransmitter release (Shen and Thayer, 1999; Takahashi and Linden, 2000), of which glutamate is the most ubiquitous.

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Cannabinoids are known to bind two G protein-coupled 7-transmembrane spanning receptors, CB_1 and CB_2 (Matsuda et al., 1990; Munro et al., 1993). Cannabinoid receptor CB_1 is the type preferentially expressed in brain and is known to mediate the psychoactive effects of cannabinoids. The classic tetrad Δ^9-tetrahydrocannabinol-induced behaviors; ataxia, catalepsy, analgesia and hypothermia show susceptibility to block by the selective cannabinoid CB_1 receptor antagonist, pyrazole compound, *N*-(piperidin-1-yl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamidehydrochloride (SR141716A) (Reche et al., 1996; Fields and Meng, 1998; Smith et al., 1998). Similarly, cannabinoid effects on excessive neuronal excitability, in vitro, are inhibited by pretreatment with SR141716A (Rinaldi-Carmona et al., 1994), indicating a cannabinoid CB_1 receptor-mediated mechanism. Conversely, cannabidiol does not bind the cannabinoid CB_1 receptor with reasonable affinity (Thomas et al., 1998) and does not produce Δ^9-tetrahydrocannabinol like behaviors that are blockable by the antagonist. Although the anticonvulsant activities of Δ^9-tetrahydrocannabinol and cannabidiol in the maximal electroshock model have been recognized for many years, it has not been investigated as to whether the anticonvulsant activity of these compounds is conferred by cannabinoid CB_1 receptor activation. Furthermore, cannabimimetic compounds such as WIN 55,212-2 dampen neuronal hyperexcitability in cultured neurons (Shen et al., 1996), but have never been evaluated for their anticonvulsant activity in whole animals. Therefore, the purpose of this study was to evaluate the cannabinoid compounds Δ^9-tetrahydrocannabinol, cannabidiol, and WIN 55,212-2 for anticonvulsant activity in the maximal electroshock model and determine if their protective activity is cannabinoid CB_1 receptor-mediated.

2. Methods

CF-1 male mice, 20–28 days old, weighing 20–30g (Harlan, Dublin, VA), were housed in the university animal facilities in groups of 4–5 for a minimum of 3 days and a maximum of 2 weeks prior to all experiments. All animals were kept in a temperature-controlled (20–22 °C) environment on a 12 h light–dark cycle (lights on at 7 am) with access to food and water ad libitum. Eight to fourteen animals were assigned to each treatment group. For anticonvulsant testing, animals received an intraperitoneal injection (i.p.) of Δ^9-tetrahydocannabinol, cannabidiol, or WIN 55,212-2 suspended in a vehicle of absolute ethanol, Emulphor-620 (Rhone-Poulenc, Princeton, NJ) and 0.9% saline at a ratio of 1:1:18. All cannabinoid compounds, as well as SR141716A, were obtained from the National Institutes of Health. All cannabinoids were administered 2 h prior to maximal electroshock. For behavioral testing, animals received an i.p. injection of Δ^9-tetrahydrocannabinol 2 h prior to testing. Phenytoin (Sigma Aldrich, St. Louis, MI), a positive control in maximal electroshock experiments, was suspended in a vehicle of polyethylene glycol and 0.9% saline at a ratio of 3:7 and was injected i.p. 30 min prior to shock. To test for antagonism of Δ^9-tetrahydrocannabinol's anticonvulsant effect, animals received an i.p. injection of SR141716A 20 min before receiving an i.p. injection of Δ^9-tetrahydrocannabinol. To test for cannabinoid CB_1 receptor-mediated effects of WIN 55,212-2 and cannabidiol, 10 mg/kg SR141716A was used, a dose found to produce maximal inhibition of Δ^9-tetrahydrocannabinol's anticonvulsant effect. Two hours following cannabinoid injection, electroshock was administered. All injections were administered at a volume of 0.1 ml/10 g (Krall et al., 1978). Appropriate vehicle controls were performed and each animal was used only once.

2.1. Maximal electroshock procedure

Maximal electroshock was produced by a 50 mA current for 0.2 s with a pulse train of 60 Hz (ECT unit model 7801, Ugo Basile, Comerio, Italy) through corneal electrodes. A drop of electrolyte solution containing lidocaine (2% lidocaine in 0.9% saline) was placed in each animal's eyes immediately prior to shock to improve electrode contact and decrease any pain localized to the eye area following shock (Swinyard et al., 1986). The shock administered was sufficient to produce hind limb extension in greater than 97% of control animals. Complete suppression of hind limb extension was considered a positive measure of anticonvulsant activity. All anticonvulsant experiments were carried out between 11:00 a.m. and 3:00 p.m. Data were expressed in terms of percent protection, that is the percentage of animals protected from hind limb extension within a treatment group. Probit analysis was used to calculate the effective dose 50% (ED_{50}) of each compound with 95% confidence limits, as well as the antagonism dose 50% (AD_{50}) of blockade Δ^9-tetrahydrocannabinol's anticonvulsant effect by SR141716A. ED_{50} was defined as the dose of drug at which 50% of the animals showed protection from hind limb extension. The effective dose 84% (ED_{84}) was defined as the dose of drug at which 84% of the animals showed protection from hind limb extension. This cannabinoid dose was used in all antagonism studies in an effort to decrease non-specific drug effects. The AD_{50} was defined as the pretreatment dose of SR141716A that abolished protection from hind limb extension in 50% of the animals treated with Δ^9-tetrahydrocannabinol. Data analysis was performed using the method of Litchfield and Wilcoxon (Litchfield and Wilcoxon, 1949). Statistical significance ($P \leq 0.05$) was determined using the Fisher Exact Test where appropriate. Dose–re-

sponse curves were generated using Microsoft Excel 97 in conjunction with Origin 6.0 software.

2.2. Behavioral testing procedures

Measurement of spontaneous activity in mice occurred in standard activity chambers interfaced with a Digiscan Animal Activity Monitor (Omnitech Electronics, Columbus, OH). A standard tail-flick apparatus (Dewey et al., 1970) and a telethermometer (Yellow Springs Instrument, Yellow Springs, OH) were used to measure antinociception and rectal temperature, respectively. Prior to testing in the behavioral procedures, mice were acclimated overnight to the experimental setting (ambient temperature 22–24 °C). Pre-injection control values were determined for rectal temperature and tail-flick latency (in seconds). Mice were injected i.p. with drug or vehicle and 1 h and 50 min later, were placed in individual activity chambers where spontaneous activity was measured for 10 min, following a 5-min acclimation period. Activity was measured as the total number of interruptions of 16 photocell beams per chamber during the 10-min test and was expressed as the percentage inhibition of activity of the vehicle group. Tail-flick latency was measured at 2 h post-injection. Maximum latency of 10 s was used. Antinociception was calculated as percent of maximum possible effect {%MPE = [(test-control latency)/(10-control)] × 100}. Control latencies typically ranged from 1.5 to 4.0 s. At 2 h and 5 min post-injection, rectal temperature was measured. This value was expressed as the difference between control temperature (before injection) and temperatures following drug administration. Each mouse was tested in each of the three procedures. Based on data obtained from numerous previous studies with cannabinoids, maximal effects of Δ^9-tetrahydrocannabinol in each procedure were estimated as follows: 90% inhibition of spontaneous activity, 100% MPE in the tail-flick procedure, and −6 °C change in rectal temperature. ED_{50}'s were defined as the dose at which half-maximal effect occurred. Graphs were generated using Sigma Plot software and were analyzed using analysis of variance (ANOVA) and Tukey Test where appropriate.

3. Results

3.1. Cannabinoid anticonvulsant activity in maximal electroshock

Fig. 1 illustrates the dose response relationships of Δ^9-tetrahydrocannabinol, WIN 55,212-2, cannabidiol and phenytoin in maximal electroshock. Each point represents data obtained from groups of 8 to 11 animals. Each drug was tested at the time of peak effect that, for each compound, was 2 h post-injection. The resulting ED_{50} values for Δ^9-tetrahydrocannabinol, WIN 55,212-2, and cannabidiol were 42, 47 and 80 mg/kg i.p., respectively. The

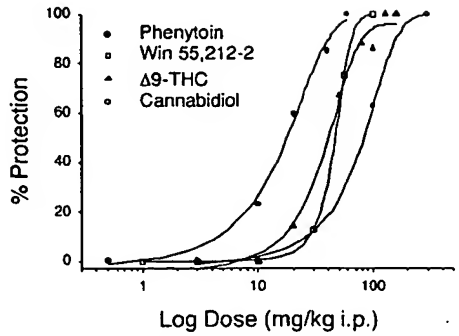

Fig. 1. Log dose response curve of Δ^9-tetrahydrocannabinol (Δ^9-THC), WIN 55,212-2, cannabidiol and phenytoin in the maximal electroshock model of seizure. Percentage of protection defined as number of animals that do not exhibit hind limb extension with electroshock ($n = 8$ per dose, per drug).

dose–response curves for Δ^9-tetrahydrocannabinol, WIN 55,212-2, and cannabidiol indicated that these compounds were equally efficacious to phenytoin in this model (ED_{50} = 18 mg/kg i.p.). Vehicle (1 Emulphor-620:1 ethanol:18 0.9% saline) showed no anticonvulsant activity in maximal electroshock as did animals treated with saline only (0.9% i.p.).

3.2. Effect of SR141716A pretreatment on cannabinoid anticonvulsant activity

The effects of SR141716A pretreatment on Δ^9-tetrahydrocannabinol's anticonvulsant activity are presented in Fig. 2. Δ^9-tetrahydrocannabinol was administered at its effective dose 84% (ED_{84}) to avoid non-specific, receptor-independent effects. With increasing pretreatment doses of SR141716A, up to 10 mg/kg, the anticonvulsant activity of Δ^9-tetrahydrocannabinol significantly decreased from 84% to 0% protection ($P \leq 0.001$ Fisher Exact Test). The AD_{50} of SR141716A was determined to be 2.5 mg/kg. Each data point represents 9–14 animals. Fig. 3 illustrates the effect of SR141716A pretreatment on the anticonvulsant activity of WIN 55,212-2 and cannabidiol when administered at their ED_{84} doses. SR141716A pretreatment completely blocked the anticonvulsant activity of the cannabimimetic WIN 55,212-2 with protection decreasing significantly from 84% to 0% ($P \leq 0.001$ Fisher Exact Test) (Fig. 3(A)). However, SR141716A failed to antagonize the anticonvulsant effect of cannabidiol significantly. The protection produced by cannabidiol in the absence and presence of SR141716A was 84% and 63%, respectively ($P = 0.067$ Fisher Exact Test) (Fig. 3(B)). SR141716A at doses up to 10 mg/kg alone had no anticonvulsant effect. A proconvulsant effect for SR141716A was not observed because the electroconvulsive threshold was not evaluated for this drug. However, the duration of tonic hind limb extension was evaluated and showed that SR141716A-treated animals were not statistically significant from vehicle-treated or control animals (data not shown). Further

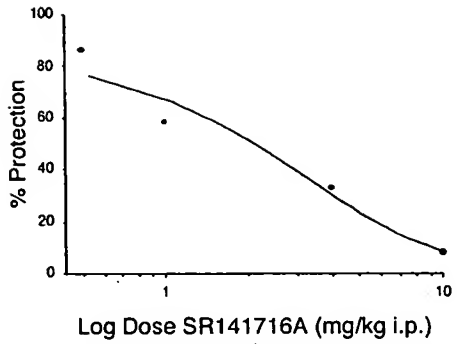

Fig. 2. Log dose inhibition curve of SR141716A in blocking the anticonvulsant effect of Δ^9-tetrahydrocannabinol. SR141716A was administered i.p. 20 min prior to the i.p. injection of Δ^9-tetrahydrocannabinol at its ED_{84} dose (70 mg/kg i.p.). Maximal electroshock was administered 2 h post Δ^9-tetrahydrocannabinol injection ($n = 9–14$ mice/group).

studies evaluating the effect of SR141716A on seizure threshold may elucidate a possible proconvulsant effect.

3.3. Effects of Δ^9-tetrahydrocannabinol on behavior

Cannabinoids produce stereotypic behaviors that include analgesia, hypothermia, and ataxia. Analgesia was measured using the tail-flick test. Spontaneous locomotor activity was quantified by the number of photocell interruptions within a 10-min time period and was expressed as the percentage inhibition of vehicle level activity. Hypothermia was measured by rectal temperature. An n of six animals per group per test was used. The results of our analysis showed that doses of Δ^9-tetrahydrocannabinol that produced anticonvulsant activity also produced behavioral effects. Fig. 4 shows the dose response relationship of

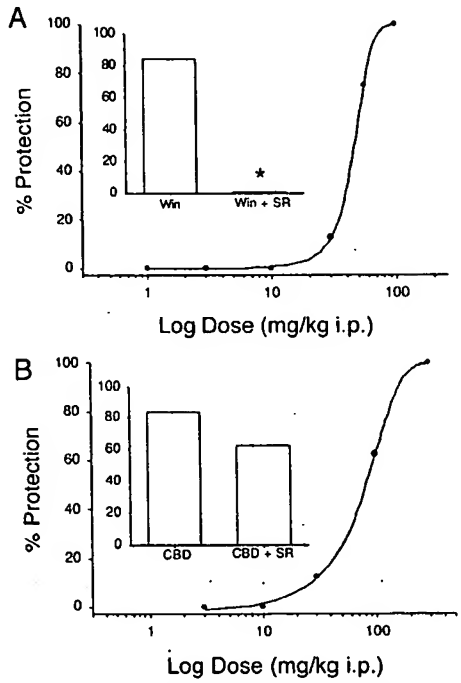

Fig. 3. Effects of SR141716A (SR) (10 mg/kg) pretreatment on anticonvulsant effects of WIN 55,212-2 (Win), or cannabidiol (CBD). The anticonvulsant log dose response curve of WIN 55,212-2 alone is presented in (A). The insert shows the effects of SR141716A (10 mg/kg i.p.) pretreatment on the ED_{84} dose (60 mg/kg i.p.) of WIN 55,212-2 ($P \leq 0.001$ Fisher Exact Test). The anticonvulsant dose response curve of cannabidiol is presented in (B). The insert shows the effects of SR141716A (10 mg/kg i.p.) pretreatment before an ED_{84} dose (160 mg/kg i.p.) of cannabidiol ($P = 0.067$ Fisher Exact Test). At least eight animals per group were tested.

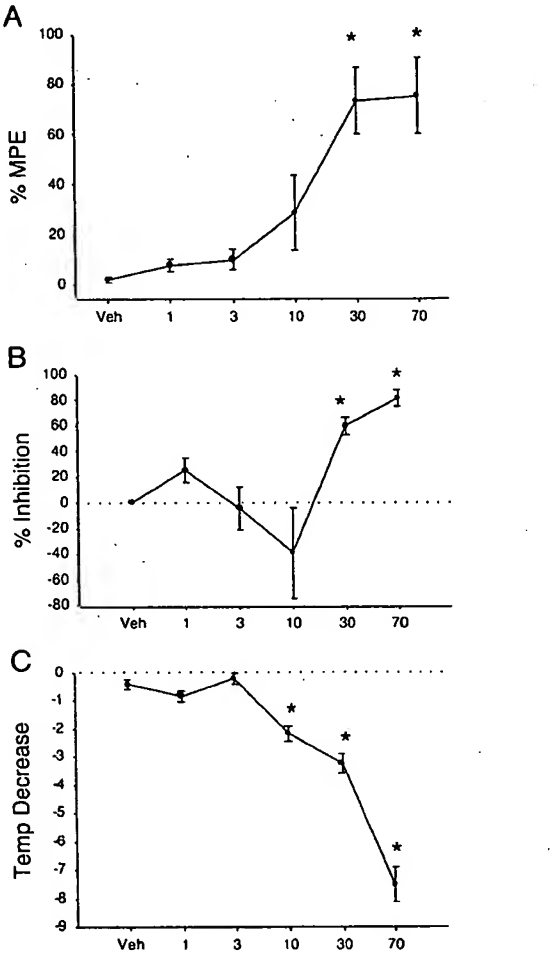

Fig. 4. Dose response of the behavioral effects of vehicle and Δ^9-tetrahydrocannabinol at 1, 3, 10, 30 and 70 mg/kg i.p. Δ^9-Tetrahydrocannabinol at 70 mg/kg i.p., the ED_{84} anticonvulsant dose, produced significant behavioral effects in the three parameters measured. (A) Dose response of Δ^9-tetrahydrocannabinol-induced analgesia as measured by the tail-flick test. Δ^9-Tetrahydrocannabinol-treated animals showed significant analgesia at 30 and 70 mg/kg compared to vehicle animals ($P \leq 0.05$ ANOVA, Tukey Test). (B) Dose response of Δ^9-tetrahydrocannabinol attenuated spontaneous activity expressed as Percentage of inhibition. Animals treated with 30 and 70 mg/kg Δ^9-tetrahydrocannabinol exhibited significantly less spontaneous activity than vehicle animals ($P \leq 0.01$, ANOVA, Tukey Test). (C) Dose response of Δ^9-tetrahydrocannabinol-induced hypothermia, measured by rectal temperature, shows that animals treated with 10, 30 and 70 mg/kg Δ^9-tetrahydrocannabinol had significantly lower body temperature compared to vehicle ($P \leq 0.05$ ANOVA, Tukey Test).

Δ^9-tetrahydrocannabinol in each of the behavioral parameters measured following a 2-h pretreatment, the time point at which maximum anticonvulsant effects were observed. Our data showed that significant levels of analgesia (ED_{50} = 11.7 mg/kg i.p.) ($P \leq 0.05$ ANOVA, Tukey Test) and a significant decrease in spontaneous activity (ED_{50} = 47 mg/kg i.p.) ($P \leq 0.01$ ANOVA, Tukey Test) were experienced at 30 and 70 mg/kg i.p. Δ^9-tetrahydrocannabinol. Significant hypothermia (ED_{50} = 16.6 mg/kg i.p.) was produced with 10, 30 and 70 mg/kg i.p. Δ^9-tetrahydrocannabinol ($p \leq 0.05$ ANOVA, Tukey Test).

4. Discussion

Studies by Karler and others demonstrated that Δ^9-tetrahydrocannabinol and cannabidiol were anticonvulsant (Karler et al., 1973, 1974; Consroe et al., 1982). These results raised the possibility that cannabinoid CB_1 receptor activation may mediate the anticonvulsant effect of cannabinoids. However, direct evidence that cannabinoid anticonvulsant effects are mediated by cannabinoid CB_1 receptor activation was not provided in these research efforts. The studies in this report provide direct evidence that, like the classic tetrad of cannabinoid behaviors, the anticonvulsant activity of Δ^9-tetrahydrocannabinol and WIN 55,212-2 in the maximal electroshock model is mediated by cannabinoid CB_1 receptor activation. From these data, we concluded that Δ^9-tetrahydrocannabinol and WIN 55,212-2, compounds that were anticonvulsant and bind the cannabinoid CB_1 receptor, lost their anticonvulsant activity when animals were pretreated with the selective cannabinoid CB_1 receptor antagonist SR141716A. Evidence that SR141716A blocks the ability of Δ^9-tetrahydrocannabinol and WIN 55,212-2 to prevent tonic hind limb extension in the maximal electroshock model indicates the involvement of the cannabinoid CB_1 receptor. Conversely, the anticonvulsant activity of cannabidiol, a compound that binds the cannabinoid CB_1 receptor extremely weakly, did not lose its protective activity when treated with SR141716A. Therefore, the anticonvulsant effects of Δ^9-tetrahydrocannabinol and WIN 55,212-2 are cannabinoid CB_1 receptor-mediated, while cannabidiol's protective effect is not. The results from these studies extend and support the original observations by Karler and others (Karler et al., 1973, 1974; Consroe et al., 1977) and indicate the involvement at the cannabinoid CB_1 receptor in mediating the anticonvulsant cannabinoid effects.

Prior to this investigation, WIN 55,212-2 had not been studied in the in vivo seizure models. However, it was shown to be effective in several in vitro seizure models. WIN 55,212-2 was shown to be anticonvulsant against seizures produced in the low-Mg^{2+} neuronal culture model of status epilepticus (Shen et al., 1996) and in stimulus-induced epileptiform discharges in the hippocampal slice preparation (Ameri and Simmet, 2000), effects that were blocked by SR141716A perfusion. Our finding that WIN 55,212-2 was protective in this model substantially expands the relevancy of existing in vitro data on this compound.

The anticonvulsant effects of cannabidiol were not mediated by cannabinoid CB_1 receptor activation. Anticonvulsant activity of cannabidiol was not inhibited by SR141716A under conditions where this cannabinoid CB_1 receptor antagonist completely blocked the anticonvulsant activity of Δ^9-tetrahydrocannabinol and WIN 55,212-2. This finding is consistent with data showing that although cannabidiol is anticonvulsant, it does not bind the cannabinoid CB_1 receptor with reasonable affinity (Thomas et al., 1998) nor does it evoke the classic tetrad of cannabinoid-induced behaviors. The anticonvulsant mechanism of cannabidiol remains unknown, but has been hypothesized to involve activation of γ aminobutyric acid(ergic) systems (Consroe et al., 1982).

It has been suggested that the mechanisms by which Δ^9-tetrahydrocannabinol and WIN 55,212-2 decrease hyperexcitability in in vitro models involve cannabinoid CB_1 receptor-modulated ion channels. Extensive molecular and pharmacological studies have shown that agonist binding to the cannabinoid CB_1 receptor activates an inhibitory G-protein, leading to decreased production of 3′, 5′ Cyclic adenosine monophosphate and thus reduced activity of the enzyme protein kinase A (Howlett, 1985; Felder et al., 1995; Hampson et al., 1995), known to modulate the activity of several ion channels. These G-protein-mediated effects on ion channels are pertussis-toxin sensitive (Matsuda et al., 1990; Mackie et al., 1995; Pan et al., 1996) and appear to alter permeability to multiple neuronal voltage-gated ion channels. These include voltage-gated Ca^{2+} channels, the G-protein coupled inward rectifier K^+ current (Mackie et al., 1995) and the A-type K^+ current (Deadwyler et al., 1993, 1995). The cannabinoid CB_1 receptor-mediated increases in rectifier and A-type K^+ currents serve to stabilize neuronal membrane potential, making the cell less likely to manifest seizure activity. In addition, cannabinoid CB_1 receptor activation produces a decrease in N and P/Q type voltage-gated Ca^{2+} currents (Pan et al., 1996). The subsequent reduction in presynaptic intracellular Ca^{2+} load causes a decrease in Ca^{2+}-dependent neurotransmitter release (Ishac et al., 1996; Gifford and Ashby, 1996; Katona et al., 1999), most notably of the neurotransmitter glutamate (Shen et al., 1996; Shen and Thayer, 1999; Kim and Thayer, 2000). Glutamate is the primary excitatory neurotransmitter of the central nervous system and elevated levels have been found in human epileptogenic foci (Leach et al., 1986). An attenuation of glutamate release would theoretically prevent seizure spread via synaptic transmission from an epileptic focus to the rest of the brain.

The abolition of hind limb extension following the administration of a convulsive current in maximal electroshock indicates that the anticonvulsant drug mechanism

impedes seizure spread. Drugs that are successful in suppressing maximal electroshock evoked hind limb extension are generally effective in treating generalized tonic–clonic and partial seizures. The prototype drug representing this classification is phenytoin. Δ^9-Tetrahydrocannabinol, WIN 55,212-2, and cannabidiol were as effective as phenytoin, used as a positive control in these studies, indicating that these cannabinoid compounds are potent anticonvulsants (Fig. 1). It is unlikely that the mechanism underlying cannabinoid anticonvulsant activity involves a general sedating effect resulting in suppression of motor activity and, therefore, impairment of tonic–clonic seizures. Animals are partially sedated at doses of Δ^9-tetrahydrocannabinol that produce suppression of hind limb extension. However, these animals still manifest all the motor activity characteristic of the clonic phase of seizure in the presence of sedative levels of Δ^9-tetrahydrocannabinol. In addition, post-shock animals treated with cannabinoid drugs run away immediately following cessation of clonus. This behavior indicates that the anticonvulsant activity of cannabinoid drugs is not simply due to sedation and impairment of locomotor activity. Sedation and impairment of locomotor activity does not necessarily confer anticonvulsant activity. For example, opiates are highly sedating but are not anticonvulsant in maximal electroshock. Cannabinoid compounds also produce hypothermia. However, it is not likely that this hypothermic effect is involved in the anticonvulsant action. Lowering animal body temperature to the level produced by cannabinoid drugs is not protective in the maximal electroshock model (Karler et al., 1974).

The ability to develop cannabinoids that have anticonvulsant effects, but have less psychoactive effects, may be useful in the clinical treatment of epilepsy. Unfortunately, the psychoactive side effects of Δ^9-tetrahydrocannabinol and WIN 55,212-2 limit their actual therapeutic utility. As our behavioral data reflects, at anticonvulsant doses, Δ^9-tetrahydrocannabinol produced a significant decrease in spontaneous activity, a psychoactive side effect. Likewise, cannabinoid-induced disruption of short-term memory via inhibition of long-term potentiation and long-term depression in the hippocampus could be detrimental in terms of patient function and compliance. Nevertheless, these data provide strong evidence that the anticonvulsant activity of Δ^9-tetrahydrocannabinol and WIN 55,212-2 are cannabinoid CB_1 receptor-mediated, whereas the protective activity of cannabidiol is not. These data further call into question the role cannabinoid CB_1 receptors play in the brain's ability to modulate synaptic activity, suggesting that perhaps a malfunctioning of the endogenous cannabinoid system contributes to the pathophysiology of epilepsy.

It is well-established that the hippocampus is a major region in the brain for modulating seizure activity and is especially sensitive to the development of recurrent seizure discharge or epilepsy. The high number of cannabinoid CB_1 receptors in hippocampus (Herkenham et al., 1990; Matsuda et al., 1990; Tsou et al., 1998) further implicate a role for cannabinoid CB_1 receptors and their endogenous ligands, anandamide and 2-arachidonylglycerol, in modulating excitability of the hippocampus. These data strongly suggest a role for the cannabinoid CB_1 receptor in modulating intrinsic neuronal excitability.

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References

Adams, I.B., Martin, B.R., 1996. Cannabis: pharmacology and toxicology in animals and humans. Addiction 91, 1585–1684.

Ameri, A., Simmet, T., 2000. Effects of 2-arachidonylglycerol, an endogenous cannabinoid, on neuronal activity in rat hippocampal slices. Naunyn-Schmiedeberg's Arch. Pharmacol. 361, 265–272.

Consroe, P., Jones, B., Laird, H., Reinking, J., 1977. Anticonvulsant–convulsant effects of delta-9-tetrahydrocannabinol. In: Cohen, S., Stillman, R.C. (Eds.), The Therapeutic Potential of Marihuana. Plenum Medical Book, New York, pp. 363–3821976, QV 109 T398 1975.

Consroe, P., Benedito, M.A., Leite, J.R., Carlini, E.A, Mechoulam, R., 1982. Effects of cannabidiol on behavioral seizures caused by convulsant drugs or current in mice. Eur. J. Pharmacol. 83, 293–298.

Deadwyler, S.A., Hampson, R.E., Bennett, B.A., Edwards, T.A., Mu, J., Pacheco, M.A., Ward, S.J., Childers, S.R., 1993. Cannabinoids modulate K^+ current in cultured hippocampal neurons. Recept. Channels 1, 121–134.

Deadwyler, S.A., Hampson, R.E., Mu, J., Whyte, A., Childers, S., 1995. Cannabinoids modulate voltage sensitive K^+ A-current in hippocampal neurons via a cAMP-dependent process. J. Pharmacol. Exp. Ther. 273, 734–743.

Dewey, W.L., Harris, L.S., Howes, J.F., Nuite, J.A., 1970. The effect of various neurohumoral modulators on the activity of morphine and the narcotic antagonists in the tail-flick and phenylquinone tests. J. Pharmacol. Exp. Ther. 175, 435–442.

Felder, C.C., Joyce, K.E., Briley, E.M., Mansouri, J., Mackie, K., Blond, O., Lai, Y., Ma, A.L., Mitchell, R.L., 1995. Comparison of the pharmacology and signal transduction of the human cannabinoid CB_1 and CB_2 receptors. Mol. Pharmacol. 48, 443–450.

Fields, H.L., Meng, I.D., 1998. Watching the pot boil [news]. Nat. Med. 4, 1008–1009.

Gifford, A.N., Ashby, C.R.J., 1996. Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55212-2, and is potentiated by the cannabinoid antagonist, SR 141716A. J. Pharmacol. Exp. Ther. 277, 1431–1436.

Hampson, R.E., Evans, G.J, Mu, J., Zhuang, S.Y., King, V.C., Childers, S.R., Deadwyler, S.A., 1995. Role of cyclic AMP dependent protein kinase in cannabinoid receptor modulation of K^+ "A-current" in cultured rat hippocampal neurons. Life Sci. 56, 2081–2088.

Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., De Costa, B.R., Rice, K.C., 1990. Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. U. S. A. 87, 1932–1936.

Hollister, L.E., 1983. Cannabis: finally a therapeutic agent? Drug Alcohol Depend. 11, 135–145.

Howlett, A.C., 1985. Cannabinoid inhibition of adenylate cyclase. Biochemistry of the response in neuroblastoma cell membranes. Mol. Pharmacol. 27, 429–436.

Ishac, E.J., Jiang, L., Lake, K.D., Varga, K., Abood, M.E., Kunos, G., 1996. Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB_1 receptors on peripheral sympathetic nerves. Br. J. Pharmacol. 118, 2023–2028.

Karler, R., Cely, W., Turkanis, S.A., 1973. The anticonvulsant activity of cannabidiol and cannabinol. Life Sci. 13, 1527–1531.

Karler, R., Cely, W., Turkanis, S.A., 1974. Anticonvulsant properties of delta 9-tetrahydrocannabinol and other cannabinoids. Life Sci. 15, 931–947.

Katona, I., Sperlagh, B., Sik, A., Kafalvi, A., Vizi, E.S., Mackie, K., Freund, T.F., 1999. Presynaptically located CB_1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J. Neurosci. 19, 4544–4558.

Kim, D.J., Thayer, S.A., 2000. Activation of CB_1 cannabinoid receptors inhibits neurotransmitter release from identified synaptic sites in rat hippocampal cultures. Brain Res. 852 (2), 398–405, Jan. 10.

Krall, R.L., Penry, J.K., White, B.G., Kupferburg, H.J., Swinyard, E.A., 1978. Antiepiletic drug development: II. Anticonvulsant drug screening. Epilepsia 19, 409–428.

Leach, M.J., Marden, C.M., Miller, A.A., 1986. Pharmacological studies on lamotrigine, a novel potential antiepileptic drug: II. Neurochemical studies on the mechanism of action. Epilepsia 27, 490–497.

Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose effect experiments. J. Pharmacol. Exp. Ther. 96, 99–113.

Mackie, K., Lai, Y., Westenbroek, R., Mitchell, R., 1995. Cannabinoids activate an inwardly rectifying K^+ conductance and inhibit Q-type Ca^{2+} currents in AtT20 cells transfected with rat brain cannabinoid receptor. J. Neurosci. 15, 6552–6561.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561–564.

Munro, S., Thomas, K.L., Abu-Shaar, M., 1993. Molecular characterization of a peripheral receptor for cannabinoids. Nature 365, 61–65.

Pan, X., Ikeda, S.R., Lewis, D.L., 1996. Rat brain cannabinoid receptor modulates *N*-type Ca^{2+} channels in a neuronal expression system. Mol. Pharmacol. 49, 707–714.

Reche, I., Fuentes, J.A., Ruiz-Gayo, M., 1996. Potentiation of delta 9-tetrahydrocannabinol-induced analgesia by morphine in mice: involvement of mu-and kappa-opioid receptors. Eur. J. Pharmacol. 318, 11–16.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Shire, D., Calandra, B., Congy, C., Martinez, S., Maruani, J., Neliat, G., Caput, D., 1994. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 350, 240–244.

Shen, M., Thayer, S.A., 1999. Delta9-tetrahydrocannabinol acts as a partial agonist to modulate glutamatergic synaptic transmission between rat hippocampal neurons in culture. Mol. Pharmacol. 55, 8–13.

Shen, M., Piser, T.M., Seybold, V.S., Thayer, S.A., 1996. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. J. Neurosci. 16, 4322–4334.

Smith, F.L., Fujimori, K., Lowe, J., Welch, S.P., 1998. Characterization of delta9-tetrahydrocannabinol and anandamide antinociception in nonarthritic and arthritic rats. Pharmacol. Biochem. Behav. 60, 183–191.

Swinyard, E.A., Sofia, R.D., Kupferberg, H.J., 1986. Comparative anticonvulsant activity and neurotoxicity of felbamate and four prototype antiepileptic drugs in mice and rats. Epilepsia 27, 27–34.

Takahashi, K.A., Linden, D.J., 2000. Cannabinoid receptor modulation of synapses received by cerebellar Purkinje cells. J. Neurophysiol. 83, 1167–1180.

Thomas, B.F., Gilliam, A.F., Burch, D.F., Roche, M.J., Seltzman, H.H., 1998. Comparative receptor binding analyses of cannabinoid agonists and antagonists. J. Pharmacol. Exp. Ther. 285, 285–292.

Tsou, K., Brown, S., Sanudo-Pena, M.C., Mackie, K., Walker, J.M., 1998. Immunohistochemical distribution of cannabinoid CB_1 receptors in the rat central nervous system. Neuroscience 83, 393–411.

Zarrelli, M.M., Beghi, E., Rocca, W.A., Hauser, W.A., 1999. Incidence of epileptic syndromes in Rochester, Minnesota: 1980–1984. Epilepsia 40, 1708–1714.